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ABSTRACT

This publication encourages student involvement in biological research through student research with the cooperation of teachers and scientists. The contents of the book are divided into two sections. The first section introduces students to research investigations and includes: (1) "How the Investigations Are Set Up and the Rationale Behind Their Designs"; (2) "Background on the Writing Partnerships between the High School Teachers and Scientists"; (3) "Some Practical Points about Experimental Design and Open-Ended Investigations"; (4) "Safety Issues Involved in Student Research"; (5) "General Guidelines for Supervising Student Research"; (6) "Where to Find Mentors and the Role They Play in Student Research"; (7) "Where to Find Student Research Opportunities"; (8) "Modifying These Investigations for Students Who Are Exceptional"; and (9) "How the Student Research Investigations Address Assessment and Standards". The second section focuses on student research core laboratory investigations with variations and includes: (1) "A Study of Allelopathy in Plants"; (2) "Biosphere 3--It's a Small World After All"; (3) "Tolerance Testing with a Hairless "Guinea Pig"; (4) "A Community Underfoot: Density and Diversity of Invertebrates in Soil or Ground Cover"; (5) "Isolation of Nutritional Mutants of E.Coli"; (6) "Which Way to Grow: Exploring Tropic Responses of Plants"; (7) "The Impact of Mollusks on Water Quality"; (8) "Plant Tissue Culture: Embryo Isolation and Tissue Culture Initiation"; (9) "A Study of Snail Behavior"; (10) "Stressed Out Plants"; and (11) "Nature's Early Warning Systems". Appendices include: (1) "Writing Format for Student Research"; (2) "Resources on the NABT Web Site"; (3) "Standard Microbiological Practices and Aseptic Techniques"; (4) "Tips for Conducting Multiple Student Research Activities"; (5) "Competitions for Student Research Activities"; and (6) "Student Research Evaluation Form". (YDS)

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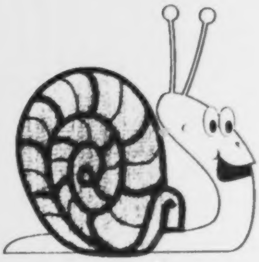

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ENCOURAGING STUDENT BIOLOGICAL RESEARCH *through* TEACHER-SCIENTIST PARTNERSHIPS

Scientists are masters of precision.

Teachers are masters at clarity.

And students are masters at spontaneity.

—Daniel Barstow, 1996.

ABOUT SAFETY

The safety procedures included with each investigation were written to assist teachers in conducting the *Student Research* investigations with their students in precollege classrooms. While every effort has been made to anticipate questions and situations that could arise, the safe implementation of these investigations must depend on the good judgment of teachers and is the responsibility of the local school district/institution. We suggest that teachers consult state or local safety manuals or textbooks for additional information. In some schools, it may be necessary to obtain special permission to do some of these investigations with students, since they involve certain organisms. We suggest that teachers check with their principal, science supervisor, scientific research review board, and/or school board to see what procedures may be required.

NABT recognizes the pervasive social phenomenon of litigation with respect to even the most unfounded claims. For that reason, NABT disclaims any legal liability for claims arising from the use of these investigations. This information has been provided to teachers and schools as a service to the profession and we provide this material only on the basis that NABT has no liability with respect to its use. Responsibility for use of any of this information is assumed by the local district/institution.

NABT believes that under the guidance of a properly trained and responsible teacher all of the *Student Research* investigations can be conducted safely in high school classrooms.

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Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation or the National Association of Biology Teachers.

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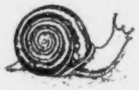

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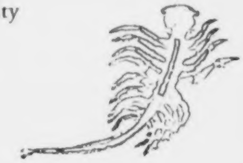

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Introducing the Student Research Investigations

• *How the Investigations Are Set Up and the Rationale Behind Their Design*

Each investigation has a Core Experiment with up to ten variations. To show the relationship between the Core Experiment and the Variations, check the first page of each lab. Here you will find an At-A-Glance Map of how each variation relates to the Core Experiment. The writing format for the investigations and a brief description of each section are included where appropriate in Appendix A. Each activity is divided into two sections— Directions for Teachers and Directions for Students. The Directions for Teachers section contains a wealth of information for the teacher when conducting student research such as content background, teaching tips, time considerations when preparing and conducting the investigation, instructional strategies, suggested readings, common student misconceptions, sample data, interpretation of the data, and sample questions with answers. The Directions for Students section is a guide for students to conduct their own research under the guidance of their teacher.

Each Core Experiment and its Variations is dynamic and is intended to be a springboard for you, the user of this material, to launch your students into the exciting world of scientific research.

• *Background on the Writing Partnerships Between the High School Teachers and Scientists*

Recruitment of teachers was done by project staff through visits to school districts and mailings to NABT members and science supervisors of public and private school districts in North Carolina, Northern Virginia, Washington, DC, and Maryland. Sites at which teachers were recruited were specifically chosen to include students of diverse cultural, ethnic, and socioeconomic backgrounds. In many cases, teachers interested in participating in the project applied with scientists with whom they had worked previously. This arrangement was—and is—the most successful. Already established teams have the rapport necessary for a smooth working relationship. In other cases, teachers were paired by site facilitators and NABT staff with scientists with whom they had not worked previously. All teacher and scientist partners were required to work together during the application process to develop an idea for a laboratory experiment. This provided the opportunity to establish rapport before the summer work began.

Twelve teams were selected from the Central North Carolina area and the Greater Metropolitan Washington, DC area. Originally, each team consisted of two teachers and one scientist who worked together to develop an idea for a laboratory experiment. At the conclusion of the lab development, two teams consisted of one teacher and one scientist, and a third team had two teachers. In the latter case, one teacher had done scientific research previously. Attrition was due to change in work responsibilities and job relocation.

During three-day workshops held at the North Carolina and the District of Columbia sites, the teams were updated by experts in the field on topics such as computer use, the process of scientific research, guidelines for supervising student research, modifying investigations for students who are exceptional, scientific writing, literary research and review, laboratory safety, and pedagogical strategies. At the conclusion of these workshops, the teams formed working schedules for a period of 30 days over the summer to develop their respective lab topics for pilot testing in the teachers' classrooms the following school year. Site visits were made by the NABT staff to each lab site during the summer and to each school during the following school year to assess the progress of the development.

In Year II, a debriefing of the 12 teams was held on the campus of North Carolina State University. Teams presented their labs and findings to each other and shared successes and difficulties they encountered during development and pilot testing of their projects. In addition, teams received workshop training so that they could present their findings to teachers in their school districts and, where possible, nationally. The labs were modified to include the input of other team members. At this time, it was decided that fewer than half were ready for national field testing and another year of development was necessary for the remaining labs. In all, the labs were field-tested over a three-year period. In the last year, all labs were field-tested specifically with students who are exceptional.

The development of this project reflects the ongoing process of science. Each Core Experiment and its Variations is dynamic and is intended to be a springboard for you, the user of this material, to launch your students into the exciting world of scientific research. Those teachers and scientists involved have shown a commitment to science and science teaching that is exceptional through their extraordinary efforts and dedication throughout this process. Every Core Experiment and its Variations is the result of years of development and effort to provide the best science and pedagogy for biological scientific research. The project participants understand that this is a process and that the findings in this document will change over time with use. Each teacher who uses these investigations has the benefit of the participants' time and knowledge of this project.

• *Some Practical Points About Experimental Design and Open-Ended Investigations*

There are many sources of information for experimental design. An excellent resource that should be part of every biology teacher's library is *Students and Research* by Julia H. Cothron, Ronald N. Giese, and Richard J. Rezba. First published in 1989 and commonly known as the "Red Book," *Students and Research* contains a wealth of information on basic and advanced principles of experimental design and analysis and strategies for managing effective student research. This publication is available from Kendall/Hunt Publishing Company. ISBN: 0-8403-7766-5.

• *Safety Issues Involved in Student Research*

There are many guidelines for laboratory safety. Establishing a safe environment is paramount to the responsibilities of the teacher and his/her institution and to the successful, safe experience of the student. The most effective way to establish a safe environment is for the teacher to model safe conduct in the laboratory and to set clear expectations of students and their behavior in the laboratory. The goal of laboratory safety is to prevent an accident. The Tips for a Safe Laboratory Environment, located in the sidebar, while not inclusive, are some common measures that you can take to establish a safe laboratory environment.

TIPS FOR A SAFE LABORATORY ENVIRONMENT

- Know how to read and interpret Material Safety Data Sheets (MSDS). Have one on file for each chemical in the laboratory.
- Know how to read and interpret the information and symbols on chemical labels.
- Know how to store, handle, dispose of, and inventory each chemical that is being used in the laboratory.
- Take a laboratory safety class.
- Plan well ahead.
- Maintain a clean and organized laboratory.
- Anticipate student accidents.
- Never leave students alone in the laboratory.
- Identify and review the location and correct use of safety devices within the laboratory and procedures on a regular basis.
- Have the proper safety equipment. If you do not have it, do not do the lab.
- Always wash hands before and after the lab.
- Clean up spills immediately.
- Provide time for proper lab cleanup.
- Have a phone in the laboratory.
- Post phone numbers for poison control, fire, police, and hospital beside the laboratory phone.
- Know the location of the master shutoff for gas, electricity, and water.
- Clearly label all paper, glass, and chemical waste containers.
- Use alcohol thermometers in place of mercury ones.
- Practice fire and evacuation drills on a regular basis.
- Give clear instructions that relate risks and demonstrate techniques for students.
- Expect exemplary behavior. Do not tolerate unsafe behavior.
- Expect students to have and wear the necessary safety equipment.
- Form and enforce written contractual agreements between the student and the teacher that clearly define safe laboratory behavior and expectations.
- Instruct and review with students the safe handling of equipment, materials, and organisms.
- Do not permit students to eat, drink, chew gum, apply cosmetics, or wear contacts in the laboratory.

TEACHER'S NOTES

• *General Guidelines for Supervising Student Research*

General Considerations for Teachers Overseeing Student Research

- Insure that students select a project that is "doable" in the given amount of time.
- Allow sufficient time for students to write up their results and prepare a display for science competitions.
- Set deadlines for students, and hold them accountable.
- Help students initially choose a project that has a good chance of giving a definite "yes" or "no" answer. If, for example, techniques involved are too difficult for the particular students to perform, the data may be inconclusive and students will become frustrated.
- Make students aware of science competitions—local, regional, state, and national—early in the year so that they can plan their research around it.
- Schedule frequent, periodic, individual meetings with students.

Time Line Sample

One important consideration when doing student research is developing a time line that is achievable with expected goals. A possible schedule for student research follows:

Week 1: Introduce experimental design and how to select a topic.

Week 2: Topic due. Begin to make entries of topic considerations into a science journal/log.

Week 3: Topic approved by teacher; begin literary search; record all information in journal.

Week 4: Hypothesis due. Literary search continues.

Week 5: Hypothesis returned; select a mentor; begin research plan.

Week 7: Research plan due; mentor's name and contact information due; all protocol forms due; Animal Review Board (ARB) and Internal Review Board (IRB) paperwork due, if applicable.

Week 8: Research plan returned and research begins if no paperwork is required.

Week 9: Paperwork approval received, if appropriate.

Week 10: Research plan with paperwork submitted to regional Science Review Committee (SRC).

Week 11: SRC returns papers. Research begins.

Week 12: Data check.

Week 16: Data check.

Week 20: Rough draft of scientific paper due.

Week 21: Abstract due.

Week 24: Final copy of scientific paper due.

Week 25: Preparation of display of scientific findings.

Week 27: Presentation of findings to the class and/or entrance into scientific competitions such as science fairs and junior academy.

Topic Selection (Source: Kathy Gotshall, Melbourne H.S., Melbourne, FL)
For most students, selecting the topic and developing a sound question are the "hardest" parts of the science research. A few weeks should be spent in general reading, browsing, and brainstorming. The topic may change several times. Encourage the students not to become discouraged. Topic selection is mainly a matter of pinpointing what interests them in science. When selecting a topic, students should take the following into consideration:

- Be certain that the topic will be interesting and doable within the allotted time period.
- Design an experiment that will contribute to science by exploring an aspect of a problem not considered before, refuting a past experiment, carrying a past experiment further, studying some natural event by finding a cause-effect relationship, or adding to the general store of scientific knowledge.
- Plan to use inexpensive and available equipment and resources.
- Choose a topic that is challenging, but that is not so difficult that one feels defeated before he/she begins. Questions like the following will help define areas of interest.
    * What is the most interesting science course you have ever taken? Why?
    * What is the last science museum you visited? What exhibit do you remember best?
    * What are your hobbies or is there a hobby that you would be interested in starting? In what ways could you incorporate your hobby into the project?
    * Have you decided on a career? If so, what is it?
    * Do you have a job? What is it?
    * Look at the list of categories of biology. Which three in order interest you the most? The least?
    * Have you done a science project before? Is there room for a further experiment?

Literary Search (Source: Author Unknown)
The literary search provides information on the topic selected and helps the researcher to become knowledgeable about the topic, what research has already been done, and what questions have been formulated from previous research. When doing research, students should be aware of the sources of information—primary or original sources of information, secondary sources or distillations of the original work, and tertiary sources that usually index or refer to the original work and distill the work even further. Primary sources include journals, experimental results, theses, summaries, monographs, interviews, questionnaires, memoirs, manuscripts, meeting reports, patents, and diaries. Secondary sources include abstracts, reviews, and journal indexes. Examples of tertiary sources include abstract indexes, author indexes, subject indexes, and formula indexes.

"How does one derive the most from a literary search?" Begin with a primary, secondary, or tertiary source to develop the topic. Remember that "searching" is an art. No precise methods can be spelled out because the material often is not listed verbatim in the way you want it. A certain amount of imagination and creativity is required to think of useful key words or nomenclature. A broad view of the project's subject area and of the project's purpose while searching the literature often will produce serendipity or eliminate the need for additional surveys.

TEACHER'S NOTES

You may wish to introduce students to a literature search by providing the following:

The Literature Search

You now have a tentative topic. You have read a book, done an experiment, or heard a discussion about your chosen topic, and now you want to get started.

Here's How To Start

1. Write down the aspects, methods, and key words that prompted you to select your topic.
2. Obtain a dictionary, handbook, and an encyclopedia to define terms and expand your original key word list. This will give you a clue or orientation as to further avenues for exploration.
3. Find a recent textbook appropriate to the topic. Use the glossary to find the pages where your key words are contained. These pages will give you access to more key words, names of authors, and lists of articles of interest. You also may conduct this search on-line, but be very selective with the sources that you find.
4. An annual review also should be consulted if your topic focus is still vague or too nebulous.
5. Consult the appropriate abstracts. These may be found on-line. Abstracts list in their indexes subject areas, authors, and journals which may be of use. Often the actual abstract provides a synopsis of the article's content (method of preparation, constants, results, and implications).
6. Your last step of this process should be accessing and reading the original literature.

As you review the information you have collected, select those articles of further interest. In all cases, the original literature will have a bibliography leading you to more information about articles or authors.

Recording Information

You will need your journal to record information from your literature search. Making a duplicate copy of your work on some other medium makes sense. You will need a separate page for each reference to keep it available at all times when you are working on your sources. Your recording system should contain a separate entry for:

- Author
- Title
- Facts of publication
- Page numbers
- Descriptive statement(s) with regard to content of the reference—also make a judgment of its usefulness
- Questions to pursue

After you have sorted through your sources and have identified those articles, books, or authors that more than likely will be included as your references, you need to:

1. Restate the article's content in your own words.
2. Use direct quotations from the reference to support, compare, or contrast your position.
3. Make critical/evaluation comments about the article.
4. Cross-reference to other articles that support or dispute this article's position.

Use this information as a guideline in your literature search and your efforts will be rewarded accurately and quickly. Good searching!

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Weekly Progress Meetings

An individual 10-minute conference may be held with each student to discuss progress to date every other week initially and monthly thereafter. A record of the meeting and plans to be completed before the next meeting can be made and a copy given to the student. This record is another means of holding students accountable. If possible, set aside a particular area of the classroom or lab specifically for research by individual students. Have students keep a journal of all work done on the research project and bring it to each meeting along with their time line for meeting the components of the research. Check the student's progress regularly. A sample form for the meeting is shown here:

Research Progress Report

Date: ______________________ Time: ____________
Name of Student: ______________________________
Name of Teacher: ______________________________

1. I have completed and am on schedule with the following tasks related to my research project at this time:

2. I have been having the following challenges accomplishing these tasks:

3. I plan to address these challenges in the following ways:

4. By the next meeting, I plan to address the following tasks:

Student Signature   Teacher Signature

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• *Where to Find Mentors and the Role They Play in Student Research*

One of the main reasons for the *Student Research* project was to develop a way for teachers to handle multiple student experiments more easily. Student research usually occurs within the classroom in conjunction with the annual school science fair. Some schools do have programs that are dedicated to student research classes, but these are rare. Research may be conducted at school or off the school campus in a research institution where students work as interns. Depending upon the researcher and the time that is available, students may conduct literary research, perform original research under the guidance of the scientist, and presentations of findings in journals and/or at scientific conferences, and/or provide lab tech support. This form of research is done out of the arena of the classroom in many cases and on the student's own time.

The other main way that research is conducted in the high school classroom is through science fairs. Students may do the research at a scientific laboratory under the guidance of a scientist or literally on the kitchen table at home. In these instances, it is not uncommon for one classroom teacher to have 120 separate experiments of various levels and content taking place. It is impossible for one person to mentor each separate activity appropriately. The role of a mentor is crucial to the success of the student's research.

Mentors may be found in all arenas from research institutions to special interest groups, such as gardening clubs. Many professional societies, two-year and four-year academic institutions, industries, hospitals, government agencies, and research institutions have education departments for facilitating student research. Locating mentors for each project can be a time-consuming process. The sharing of topics within a class can provide access to parents or individuals in their workplace who can mentor student labs. Each Core Experiment lists possible mentors specific for the lab content.

The successful mentor is one who enjoys working with youth, has a passion for science and the patience to teach and convey this passion to youth, and acknowledges his/her responsibility to give back to society for the benefit of future generations.

• *Where to Find Student Research Opportunities*

A list of sites offering opportunities for high school students in research follows. The list is by no means exhaustive, but provides an idea of the vast opportunities for students to participate in original research.

NIH Summer Internship Program in Biomedical Research: *http://www.nhlbi.nih.gov/nhlbi/train/redbook/hsnihbio.htm*. This program enables students who have a strong interest in pursuing studies related to biomedical or behavioral research, including the disciplines of biology, chemistry, physical science, psychology, computer science, biostatistics, mathematics, and biomedical engineering, to receive a research experience at the National Heart, Lung, and Blood Institute (NHLBI).

High School Extramural Programs: Research Supplement for Underrepresented Minority High School Students: *http://www.nhlbi.nih.gov/nhlbi/train/redbook/hsminsup.htm*. This program is designed to provide support for research experiences for underrepresented minorities, which includes African Americans, Hispanics, Native Americans, Alaskan Natives, and Pacific Islanders.

Research Supplements to Promote the Recruitment of Individuals with Disabilities Into Biomedical Research Careers: *http://www.nhlbi.nih.gov/nhlbi/train/redbook/hssupdis.htm*. This program provides research experiences for qualified high school students with disabilities who wish to participate in an ongoing research project during the summer months or during the school year.

NIH Summer Internship Program in Biomedical Research: *http://www.nhlbi.nih.gov/nhlbi/train/redbook/hsnihsum.htm*. The NIH Summer Internship Program in Biomedical Research is intended to give students in high school an opportunity to participate in state-of-the-art research in the intramural laboratories at the National Institutes of Health.

NINDS Summer Program in the Neurosciences: *http://www.nhlbi.nih.gov/nhlbi/train/redbook/hsnihsum.htm*. This program provides hands-on neuroscience research training for academically talented high-school students contemplating a career in biomedical research or academic medicine.

Student Career Experience Program: *http://www.nhlbi.nih.gov/nhlbi/train/redbook/hsnihsum.htm.* The Cooperative Education (COOP) is a planned and progressive, career-related student employment program.

Summer Research Fellowship Program in the Neurosciences: *http://www.nhlbi.nih.gov/nhlbi/train/redbook/hsnihsum.htm.* The NINDS Summer Research Fellowship Program in the Neurosciences is designed to give academically talented students a unique opportunity to acquire valuable hands-on research training and experience in the neurosciences.

High School Science Student Education Program: *http://www.mcw.edu/research/student/roads.htm.* Aimed at advanced chemistry, biology, and physics classes, this program provides experiences in the world of science at the Medical College of Wisconsin.

Mini Medical School for High School Students: *http://www.mcw.edu/research/student/roads.htm.* This eight-week course, consisting of four lectures and four corresponding laboratories, is designed for the advanced high school science student who wants to delve deeper into current biomedical topics and who may be considering a career in biomedicine and research at the Medical College of Wisconsin.

Research Opportunity for Academic Development in Science (ROADS): *http://www.mcw.edu/research/student/roads.htm.* The ROADS program allows minority high school students interested in science to serve as research fellows for eight weeks during the summer.

High School Leadership Training Programs: *http://www.ssc.org/summers/sprog.html.* The Sierra Student Coalition presents three national high school environmental leadership programs designed to train the leaders of the present and the future located in Colorado, Virginia, and Vermont.

"PPD: Biotechnology Works!": *http://www.workpage.com/g/07/283g.htm.* Recognizes the National Science Foundation's priority to facilitate the participation of persons with disabilities in mathematics and science.

Summer Apprentice Researchers at QUEST: *http://www.quest.ucsb.edu/education/ARQ/ARQ.html.* The Apprentice Researcher at QUEST (ARQ) program for high school students and teachers is supported by supplemental funds from Education and Human Resources at NSF. Participants conduct research through scientific inquiry, use sophisticated equipment to observe and analyze data, and recognize how mathematics and scientific theory apply to the labs of successful career scientists.

SEACAMPS San Diego and Hawaii: *http://www.seacamp.com/summer/summer.html.* This co-educational residential program for students who will be entering the 7th through the 12th grade in the upcoming school-year.

• *Modifying These Investigations for Students Who Are Exceptional*

In all efforts to educate youth, it is essential to accommodate all learning needs where possible. The AAAS *Barrier-Free in Brief* series is an excellent source of information on science/mathematics learning and disability. The four-booklet series is unique, succinct, and reliable. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs), light probes, and talking thermometers, calculators, and clocks. Any one or all of the booklets are available FREE from AAAS, Project on Science, Technology, and Disability, 1333 H Street, NW, Washington, DC 20008; 202.326.6630 (V/TDD); 202.371.9849 (FAX); bgoodric@aaas.org (E-mail). Lab-specific suggestions for accommodating the needs of exceptional students are found in the section entitled Suggested Modifications for Students Who Are Exceptional for each Core Experiment, and where appropriate in each Variation of the Core Experiment. Students already may be able to provide their own modifications. Be sensitive to the student and his/her needs and discuss these issues with him/her before suggesting any modifications. The modifications suggested here do not begin to address all the needs students with single and multiple special needs may have when conducting these investigations, but should provide suggestions to accommodate some.

• *How the Student Research Investigations Address Assessment and Standards*

Today, accountability is a function imperative in education. Assessment of what is learned and standards for that learning provide accountability for the processes and content of each learning environment. The contents, skills, and objectives of *Student Research* have been correlated with the software package *Curriculum Orchestrator* produced by *MediaSeek*. This software package has a science knowledge base that links the *Student Research* publication to national and state standards as well as other publications and various media. It is available from *MediaSeek* Technologies, Inc., 2211 Rimland Drive, Suite 224, Bellingham, WA 98226-5662, 800.372.3277. The *Student Research* labs seek to involve students actively and intelligently in the process of scientific investigation. The active involvement of students in scientific investigations is the essence of the science education standards. The investigations have been aligned with the *National Science Education Standards* (*NSES*) developed by the National Research Council and the *New Standards™ Student Performance Standards* developed by the National Center on Education and the Economy. Each set of standards has been placed in a matrix. A check mark indicates which activity is aligned with the appropriate standard for each document.

NATIONAL SCIENCE EDUCATION STANDARDS	A Study of Allelopathy in Plants	Biosphere 3—It's a Small World After All	Tolerance Testing With a Hairless "Guinea Pig"	A Community Underfoot	Isolation of Nutritional Mutants of *E. coli*	Which Way To Grow	The Impact of Mollusks on Water Quality	Plant Tissue Culture	A Study of Snail Behavior	Stressed Out Plants	Nature's Early Warning Systems
Unifying Concepts and Processes											
Systems, order, and organization		X		X			X				
Evidence, models, and explanation	X	X	X	X	X	X	X	X	X	X	X
Change, constancy, and measurement	X	X		X	X	X	X	X	X	X	X
Evolution and equilibrium		X	X	X	X		X			X	X
Form and function	X	X	X	X		X	X	X	X	X	X
Science as Inquiry											
Abilities necessary to do scientific inquiry	X	X	X	X	X	X	X	X	X	X	X
Understanding about scientific inquiry	X	X	X	X	X	X	X	X	X	X	X
History and Nature of Science											
Science as a human endeavor	X	X	X	X			X				
Nature of science	X	X	X	X	X	X	X		X	X	X
History of science	X			X			X				
Physical Science											
Properties and changes of properties in matter											
Transfer of energy		X									
Life Science											
Structure and function in living systems	X		X	X	X	X	X	X	X	X	X
Reproduction and heredity		X			X		X	X			
Regulation and behavior	X		X		X	X	X	X	X	X	X
Populations and ecosystems	X	X	X	X	X		X	X		X	X
Diversity and adaptations of organisms	X			X	X		X	X	X	X	X
Earth and Space Science											
Structure of the Earth system							X			X	X
Earth in the solar system											
Earth's history											
Science and Technology											
Understanding about science and technology		X			X						X
Science in Personal and Social Perspectives											
Personal health											
Populations, resources, and environments	X	X	X	X	X		X	X	X	X	X
Natural hazards											
Risks and benefits								X			

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NEW STANDARDS™ STUDENT PERFORMANCE STANDARDS	A Study of Allelopathy in Plants	Biosphere 3—It's a Small World After All	Tolerance Testing With a Hairless "Guinea Pig"	A Community Underfoot	Isolation of Nutritional Mutants of *E. coli*	Which Way To Grow	The Impact of Mollusks on Water Quality	Plant Tissue Culture	A Study of Snail Behavior	Stressed Out Plants	Nature's Early Warning Systems
Life Science Concepts Demonstrates conceptual understanding by using concepts to explain observations and make predictions and by representing the concepts in multiple ways (through words, diagrams, graphs, or charts, as appropriate). Both aspects of understanding — explaining and representing — are required to meet this standard.											
Structure and function in living systems, such as the complementary nature of structure and function in cells, organs, tissues, organ systems, whole organisms, and ecosystems.	X	X	X	X	X	X	X	X	X	X	X
Reproduction and heredity, such as sexual and asexual reproduction; and the role of genes and environment on trait expression.					X		X				
Regulation and behavior, such as senses and behavior, and response to environmental stimuli.	X	X	X		X	X	X	X	X	X	X
Populations and ecosystems, such as the roles of producers, consumers, and decomposers in a food web; and the effects of resources and energy transfer on populations.	X	X	X	X	X		X			X	X
Evolution, diversity, and adaptation of organisms, such as common ancestry, speciation, adaptation, variation, and extinction.	X			X	X		X				
Earth and Space Sciences Concepts Demonstrates conceptual understanding by using a concept to explain observations and make predictions and by representing the concept in multiple ways (through words, diagrams, graphs, or charts, as appropriate). Both aspects of understanding — explaining and representing — are required to meet this standard.											
Structure of the Earth's system, such as crustal plate and land form; water and rock cycles; oceans; weather; and climate.											
Earth's history, such as Earth processes including erosion and movement of plates; change over time; and fossil evidence.											
Scientific Connections and Applications Demonstrates conceptual understanding by using a concept to explain observations and make predictions and by representing the concept in multiple ways (through words, diagrams, graphs, or charts, as appropriate). Both aspects of understanding — explaining and representing — are required to meet this standard.											
Big ideas and unifying concepts, such as order and organization; models, form, and function; change and constancy; and cause and effect.	X	X	X	X	X	X	X			X	X
Impact of science, such as historical and contemporary contributions and interaction between science and society.		X	X				X			X	X
Scientific Thinking Demonstrates scientific inquiry and problem solving by using thoughtful questioning and reasoning strategies, common sense, and conceptual understanding from Science Standards 1 to 4, and appropriate methods to investigate the natural world.											
Frames questions to distinguish cause and effect; identifies or controls variable in experimental and non-experimental research settings.	X	X	X	X	X	X	X	X	X	X	
Uses concepts to explain a variety of observations and phenomena.	X	X	X	X	X	X	X	X	X	X	√
Uses evidence from reliable sources to develop descriptions, explanations, and models.	X	X	X	X	X	X	X	X	X	X	√
Proposes, recognizes, analyzes, considers, and critiques alternative explanations; and distinguishes between fact and opinion.	X	X	X	X	X	X	X	X	X	X	√
Identifies a problem; proposes and implements solutions; and evaluates the accuracy, design, and outcomes of investigations.	X	X	X	X	X	X	X	X	X	X	
Works individually and in teams to collect and share information and ideas.	X	X	X	X	X	X	X	X	X	X	√
Scientific Tools and Technologies Demonstrates competence with the tools and technologies of science by using them to collect data, make observations, analyze results, and accomplish tasks effectively.											
Uses technology and tools (such as traditional laboratory equipment, video and computer aids) to observe and measure objects, organisms, and phenomena directly, indirectly, and remotely.											
Acquires information from multiple sources, such as experimentation, print, the Internet, and computer databases.	X	X	X	X	X		X				X
Scientific Communication Demonstrates effective scientific communication by clearly describing aspects of the natural world using accurate data, graphs, or other appropriate media to convey depth of conceptual understanding in science.											
Represents data and results in multiple ways, such as numbers, tables, and graphs; drawings, diagrams, and artwork; and technical and creative writing.	X	X	X	X	X	X	X	X	X	X	X
Argues from evidence, such as data produced through his or her own experiments or those done by others.	X	X	X	X	X	X	X	X	X	X	X
Critiques published materials.	X	X	X	X	X	X	X	X	X	X	√
Explains a scientific concept or procedure to other participants.	X	X	X	X	X	X	X	X	X	X	X
Communicates in a form suited to the purpose and the audience, such as writing instructions that others can follow; critiquing written and oral explanations; and using data to resolve disagreements.	X	X	X	X	X	X	X	X	X	X	X
Scientific Investigations Demonstrates scientific competence by completing projects drawn from the following kinds of investigations:											
Controlled experiment	X	X	X	X	X	X	X	X	X	X	X
Fieldwork	X			X			X				
Design	X	X	X	X	X	X	X	X	X	X	X
Secondary research, such as use of others' data	X	X	X	X	X	X	X	X	X	X	X
Non-experimental research using print and electronic information, such as journals, videos, or computers	X	X	X	X	X	X	X	X	X	X	X

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NOTES

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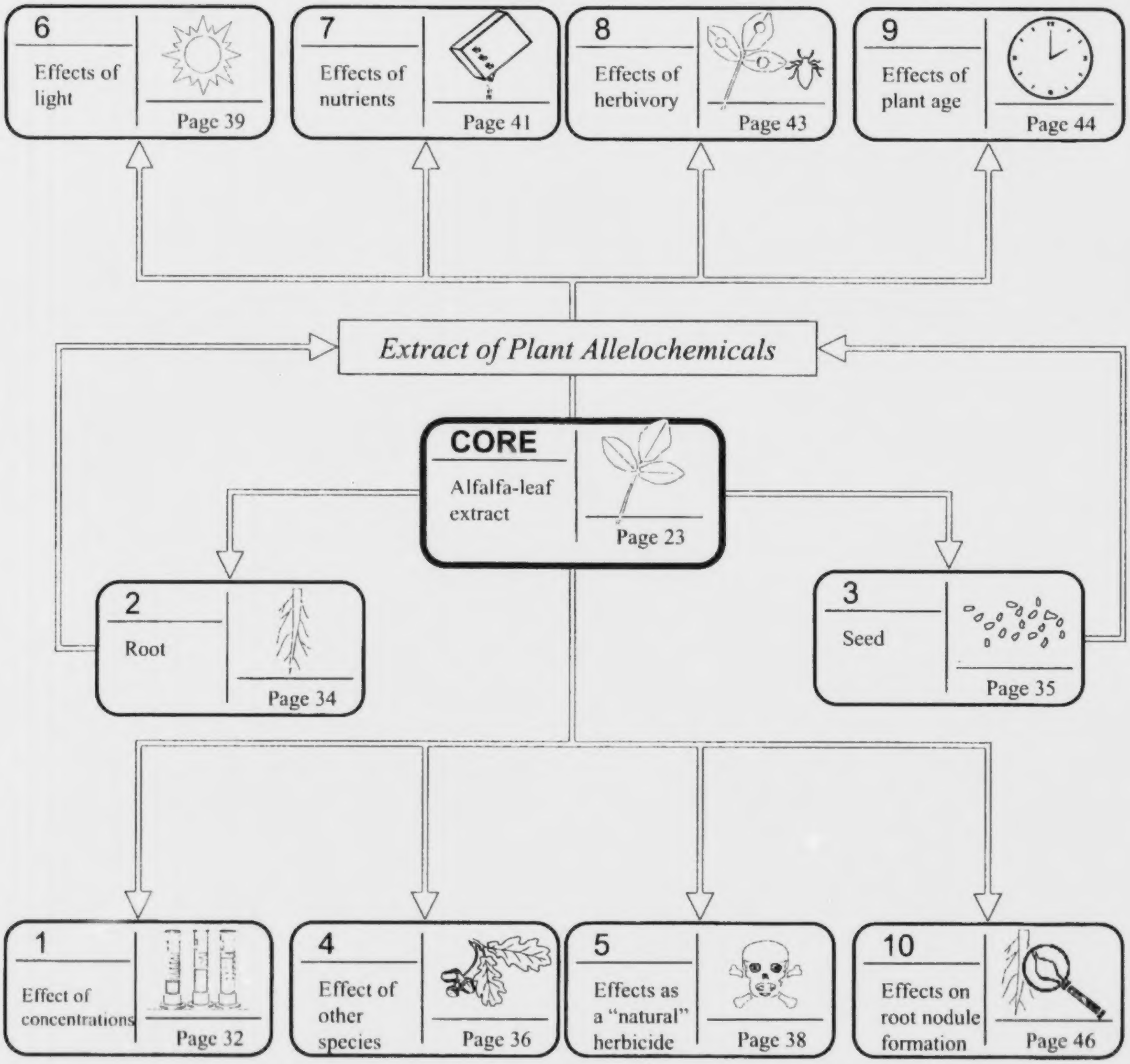
A Study of Allelopathy in Plants

At-A-Glance Map of the Core Experiment and Its Variations


6
Effects of light
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Effects of nutrients
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Extract of Plant Allelochemicals
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Effect of other species
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Effects as a "natural" herbicide
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Effects on root nodule formation
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NOTES

A Study of Allelopathy in Plants

SYNOPSIS FOR CORE EXPERIMENT

Students will test the effects of the water-soluble extract or leachate of alfalfa leaves on radish, white clover, and rye grass seeds. They will determine whether the leachate from the alfalfa leaves contains allelopathic agents that affect the germination or growth of the target seed species.

APPROPRIATE BIOLOGY LEVEL

Introductory and advanced

TEACHER PARTNER

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Washington & Lee High School
1300 North Quincy Street
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SCIENTIST PARTNER

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Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 31. For the page number of a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Identify the parts of a plant that may produce allelopathic substances.
- Describe the observable effects of allelopathy on seed germination, seedling appearance, and growth of roots and leaves.
- Discuss the ecological implications of allelopathy.

MATERIALS NEEDED

For the teacher preparation, you will need the following for a class of 24:

- 2 shallow 30 x 60-cm plastic planting trays or 18 10-cm planting pots
- 8 kg (16 lbs) topsoil or potting soil
- 100 g alfalfa seeds
- 1 balance
- 1 weigh boat
- 1 window with southern exposure or 1 fluorescent Gro-Lite™ bank with automatic timer
- 18 disposable 10 x 150-mm petri dishes
- 1 refrigerator

You will need the following for each group of four students in a class of 24:

- 5 g freshly cut alfalfa shoots
- 1 pair of scissors
- 1 150-mL glass beaker
- 50 mL distilled water
- 1 permanent marking pen
- 18 9-cm disposable, plastic petri dishes
- 6 40 x 40-cm layers of cheesecloth
- 1 50-mL flask
- 54 sheets of 8-cm filter paper, coffee filters, or white paper towel sheets
- 2 5-mL disposable, plastic pipettes or syringes without needles
- 60 seeds each of radish, white clover, and rye grass
- 18 80 x 10-mm strips of Parafilm™ (*optional*)
- 1 metric ruler
- 1 data journal

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (30 to 45 minutes)

- Prepare the alfalfa extract and petri dishes.

Day 2 (5 minutes)

- Examine the extract for color change and evidence of chemicals leaching into the water.

Day 3 (30 to 45 minutes)

- Remove alfalfa leaves from the leaf extract. Set up the control and experimental petri dishes with target seed species and either the leaf extract or distilled water.

Day 4 (10 minutes)

- Check for seed germination.

Days 5 and 7 (10 to 20 minutes)

- Observe and record the number of germinated seeds and their appearance.

Day 9 (45 minutes)

- Make final observations of seed appearance and the number of seeds that have germinated. Count the number of leaves on each seedling and measure the root and shoot lengths.

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TEACHER'S NOTES

PREPARATION TIME REQUIRED

15 days before lab (1 hour)

- Plant seeds.

1 day before lab (30 minutes)

- Locate and set out student supplies.

SAFETY PROCEDURES

Avoid plants that elicit allergies.

Wash hands after touching plants.

Students with allergies should wear nonallergenic gloves.

DIRECTIONS FOR SETTING UP THE LAB

Planting the Alfalfa

- Weigh approximately 50 g of alfalfa seeds for each plastic planting flat. Spread the seeds evenly across the soil surface and cover them with approximately 1 cm of soil.
- Water the seeds thoroughly and place them in a well-lit area. The plants should have 16 hours of daylight for optimal growth. If using natural light, a southern exposure is preferable. If artificial light is used, place the plants approximately 0.5 m (20 inches) away from the light. It is suggested that 6 120-cm (48-inch) fluorescent light bulbs be used.
- Continue to water as needed to keep the soil moist.
- Expect sprouting in 3 to 5 days. The alfalfa will be ready to harvest within 10 to 15 days.

TEACHER BACKGROUND

Content Information

Ecology is the study of interactions between organisms and their environment. One type of interaction frequently studied by ecologists is the competition between two or more species for limited resources. When a resource such as water is in limited supply, those species that need it will compete for it.

Competition may be defined as one organism having a negative effect on another by restricting its access to a resource that is in limited supply. Competition may be intraspecific, occurring between individuals of the same species, or interspecific, occurring among different species. One possible result of competition is the extinction of a species. The Russian biologist G.F. Gause demonstrated this possibility with two species of *Paramecium* (Towle, 1989). When grown together in a laboratory culture, one species always became extinct, even though each species grew well alone.

Another possible outcome of competition is specialization of each species in their use of resources. As this happens, their resource requirements no longer overlap entirely and they are able to coexist in the same environment. Competition between species can be an important factor influencing the distribution and evolution of species.

There are two general ways an organism can limit the resources available to another organism—exploitation or interference competition. In exploitation competition, one species is better adapted for using the limited resource. This indirectly deprives the other species of the resource. An example is a desert plant that has an extensive root system which allows it to use water that would otherwise be available to another plant species. The second type of competition, interference, occurs when one species directly prevents the other from using the resource by either physical or chemical means. Allelopathy is a form of chemical interference competition utilized by plants.

The word allelopathy is derived from the root words *allelon*, "of each other," and *pathos*, "to suffer." Allelopathy involves a chemical inhibition of one species by another. Molecules produced by one plant, mostly secondary metabolites, are released into the environment and then affect the growth and development of neighboring plants. In California's Mojave Desert, for example, creosote shrubs are thought to exclude burro-weed by exuding a chemical from their roots. This chemical may inhibit the root growth of the burro-weed (Dutton, 1993).

Allelopathic chemicals may be present in any part of a plant including the leaves, flowers, fruits, stems, roots, rhizomes, and seeds. These toxins can affect a target species in a number of ways. For example, they may inhibit the target species' nutrient uptake, or they may inhibit shoot or root growth. Some plants, such as clover, have a symbiotic or mutually beneficial relationship with nitrogen-fixing bacteria that provides nitrogen for the plant's growth. An allelopathic agent against clover might directly attack the symbiotic bacteria and destroy the plant's source of usable nitrogen.

Allelopathy is not a recent discovery. In 300 BC, Theophrastus realized that planting chick peas made the soil unsuitable for many other plants. Earlier records of allelopathy date back before 300 BC when Democritus reported that weeds could be controlled by using naturally occurring plant products and trees could be killed by treating their roots with a mixture of lupine flowers soaked in hemlock juice. In 1 AD, Pliny, the Roman naturalist, reported that barley and bitter vetch prevented the growth of other plants in the same soil. In more recent history, A.B. Massey (1925) determined that toxic substances released into the soil by walnut trees inhibited the growth of almost all other plants. Some researchers, however, contend that other factors, such as competition for light or nutrients, are the reason why certain plants are unable to grow together and that allelopathy is not a factor.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

Correct Concept	Misconception
• Plants can interact with other plants and their environment.	• Plants have no effect on each other.
• Plants compete for limited resources.	• Only animals compete for resources.
• Plants form allelopathic substances that can inhibit the growth of other plants.	• Plants form chemical substances only against insects.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Start by brewing a pot of tea to help introduce allelopathy. Ask the students what is creating the smell and color in the tea. Relate the smell to the chemicals in the tea plant, such as tannin.

Have some potted herbs, such as mint or pennyroyal, for students to rub between their fingers. Discuss the presence of chemicals that are responsible for the odors that they emit. Freshly cut pine boughs also work well to illustrate the point that plants contain many chemicals.

Discuss the botanical origin of many of our modern medicines. Some of these medicinal substances are derived from common plants and trees that students may have growing in their own backyards. An example is foxglove (*Digitalis purpurea*), the source of a drug used to treat some heart conditions. One of the reasons for the

TEACHING TIPS

- Store the alfalfa-leaf extract in the refrigerator to control contamination.
- If student lab time is limited, the teacher may prepare the alfalfa-leaf extract in advance. Prepare 300 mL of extract per class.
- During field testing, contamination by bacteria was sometimes a concern. The concern lies in whether the effect on the target species was truly allelopathic, therefore plant-plant interaction, or an action of bacterial contamination.
- To manage time and the large number of petri dishes, do not have each student group set up each target species. Rather, have each student group be responsible for setting up one target species group and providing their data for a class set of data.
- If time is limited, fresh alfalfa sprouts can be purchased in the produce section of many grocery stores. Be aware that ungerminated seeds and seed coats are often mixed in with the alfalfa sprouts. One container of alfalfa sprouts may not provide sufficient plant material.
- Instead of petri dishes, students may use large culture dishes with larger-sized filter paper or resealable plastic bags and paper towels. The amount of alfalfa tea and distilled water will need adjustment so that the filter paper is moist, but not oversaturated with water.
- Agricultural and garden supply stores have a good supply of a variety of seeds. Some seeds are also available through biological supply companies.
- Seeds are available from the beginning of February or from some garden supply stores throughout the year. If you anticipate doing the lab in the fall, purchase seeds during the prior spring or early summer.
- Check with students as to the types of trees and plants they may have in their yards; many, such as black walnut, mint, and ragweed, are good sources of allelopathic substances.

Weed seeds can be purchased from:

Valley Seed Service
P. O. Box 9335
Fresno, CA 93791
559.435.2163

(continued on page 24)

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TEACHING TIPS

(continued from page 23)

- Avoid overwetting the paper lining the petri dish. Paper should be damp throughout the lab with no standing water. Excess water can be poured off provided it is done for all petri dishes.

preservation of the tropical rain forests and other areas is the potential for discovery of valuable medicines.

Not all substances that plants produce are useful as medicines; many are toxins. Some chemicals produced by plants serve as defenses against predators by making the plant distasteful or toxic. The oils produced by poison ivy are a good example. Even the medicinal chemical extracted from foxglove is toxic in larger doses. Remind the students that allelopathic substances are not just found in the leaves, but also may be present in other parts of the plant, like the roots, stems, and flowers.

Discuss allelopathy as a form of interference competition and relate it to broader ecological and evolutionary principles. You could also mention the practical human applications related to agriculture.

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can develop into hypotheses.

Students should generate a hypothesis for the Core Experiment based on what they know about the production of chemicals by plants as a form of competition. They should consider how these chemicals affect other plants.

Tell the students that alfalfa sprouts and some common seeds are available for the experiment. Alfalfa sprouts are believed to have an allelopathic effect on some plants. The students need to develop a technique to remove the allelopathic chemicals from the alfalfa leaves. They probably will come up with some great ideas for drying the leaves or using chemical extraction. Remind them that water is a universal solvent and is probably the solvent most likely to occur in nature.

Sample Hypotheses

- Alfalfa-leaf extract will prevent the seeds from germinating.
- Alfalfa-leaf extract will affect the geotropic response of the germinating seeds.
- After germinating, seeds treated with alfalfa-leaf extract will not continue to grow.
- Leaf extract from alfalfa will have a negative effect on the germination and seedling height of radish, white clover, and rye grass seeds.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each group of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 30.

Question

Will alfalfa-leaf extract affect seed germination or growth?

Hypothesis

Leaf extract from alfalfa will have a negative effect on the germination and seedling height of radish, white clover, and rye grass seeds.

Rationale

Alfalfa sprouts are believed to have allelopathic effects that may affect common seeds, such as radish, clover, and rye.

Procedure

Alfalfa-Leaf Extract Preparation

1. Harvest 5 g of alfalfa shoots by cutting them close to the soil surface. See Figure 1.

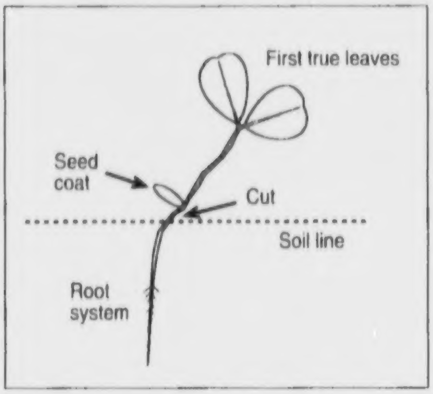


Figure 1. Alfalfa shoot.

2. Remove any loose soil from the shoots.
3. Place the alfalfa shoots in a 150-mL beaker and add 25 mL of distilled water. See Figure 2.

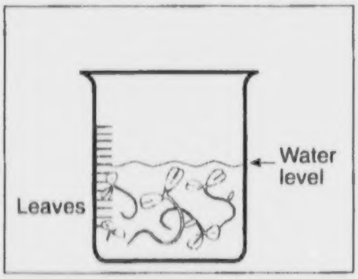


Figure 2. Beaker with submerged shoots.

4. Press the shoots down so they are submerged totally and cover the beaker with Parafilm™ or plastic wrap to prevent evaporation.
5. Let the alfalfa shoot "tea" steep for 2 days in a cool place. If the room temperature is above 22°C, place the "tea" in a refrigerator.
6. On the second day of steeping, examine the "tea" for color, odor, and consistency.
7. Label the "tea" *alfalfa-leaf extract*. If you are unable to continue with the experimental design setup at this time, store the extract at 4°C in a refrigerator. It should remain stable for a few days.
8. You will need 6 petri dishes for each target seed species—3 controls and 3 treatments. Label each petri dish with petri dish number, treatment or control, target species, date, and group initials as shown in Figure 3.

TEACHER'S NOTES

TEACHER'S NOTES

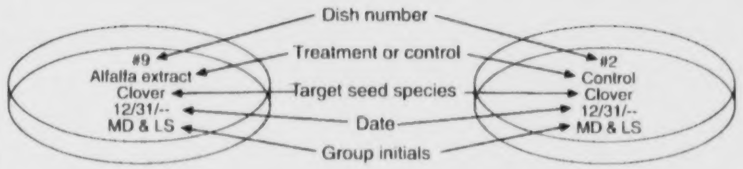


Figure 3. Labeled petri dishes.

Target Seed Setup

1. Pour the alfalfa-leaf extract through several layers of cheesecloth to remove any suspended matter and collect the liquid in a 50-mL flask. This extract will be used to treat the seeds.
2. Line each petri dish with 3 sheets of filter paper.
3. Add 5 mL of distilled water to each of the control dishes with a 5-mL disposable pipette or syringe.
4. Add 5 mL of leaf extract to each of the treatment dishes with a 5-mL disposable pipette or syringe.
5. Place 10 radish seeds in each of the 3 control petri dishes and each of the 3 treatment petri dishes. Spread the seeds evenly in the dish. Cover with the lid. If necessary, seal with Parafilm™ or tape to prevent evaporation.
6. Repeat Step 5 using the white clover and the rye grass seeds.
7. Place all the petri dishes in a warm place with filtered light. Direct sunlight may alter the chemical compounds in the extract.

Seed Observation and Data Collection

1. Check the petri dishes the following day for evidence of germination or sprouting. Record these observations as Day 1.
2. On Days 2, 5, and 7, record the number of germinated seeds and descriptions of each seedling's appearance in each dish.
3. On Day 7, also measure the shoot and root length of each seedling.

DATA ANALYSIS AND INTERPRETATION

Sample Data 1

For each of the 3 target species of radish, white clover, and rye grass, compare the experimental and control groups, with respect to the mean number of seeds germinated, the mean number of leaves produced, the mean shoot length, the mean root length, and other responses of the different target species, such as changes in color or number of leaves. Consider whether all the target species responded equally to the alfalfa-leaf extract.

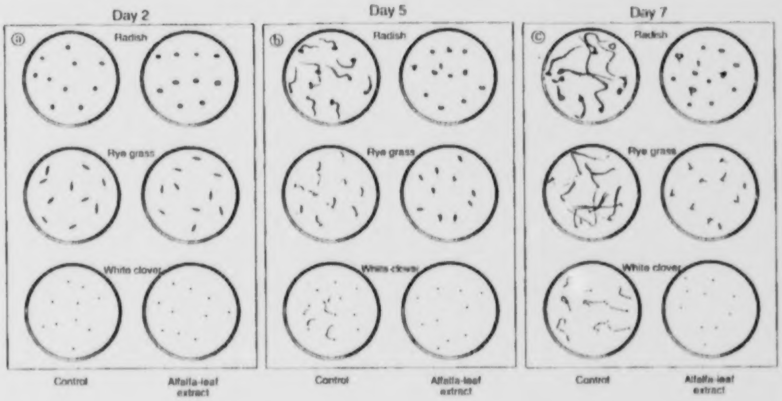
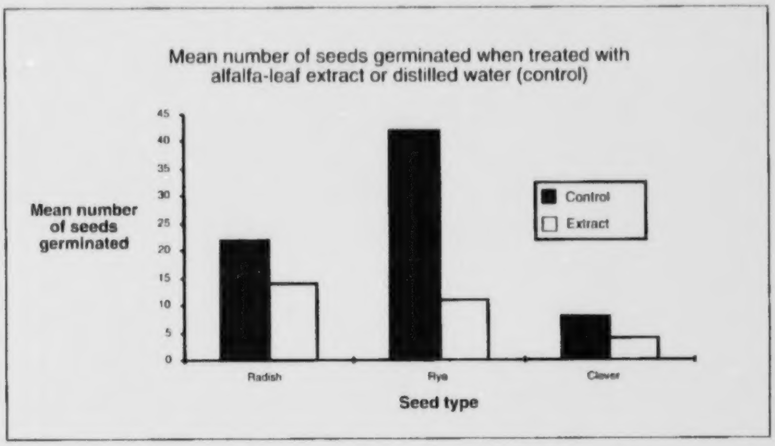

Day 2
Day 5
Day 7
ⓐ
ⓑ
ⓒ
Radish
Rye grass
White clover
Control
Alfalfa-leaf extract

Figure 4. Results of alfalfa-leaf extract treatment on rye, clover, and radish seed germination at Days 2, 5, and 7.

Sample Data 2



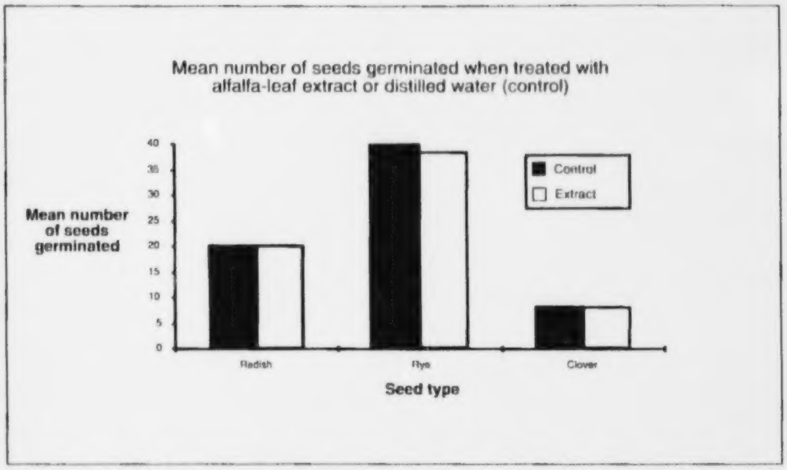
Graph A. Effects of alfalfa-leaf extract on seed germination.

TEACHER'S NOTES

Interpretation

The results support the hypothesis. For all 3 target species — radish, white clover, and rye grass — the alfalfa-leaf extract had a negative effect on the germination of the seeds.

Sample Data 3



Graph B. Effects of alfalfa-leaf extract on seed germination.

Interpretation

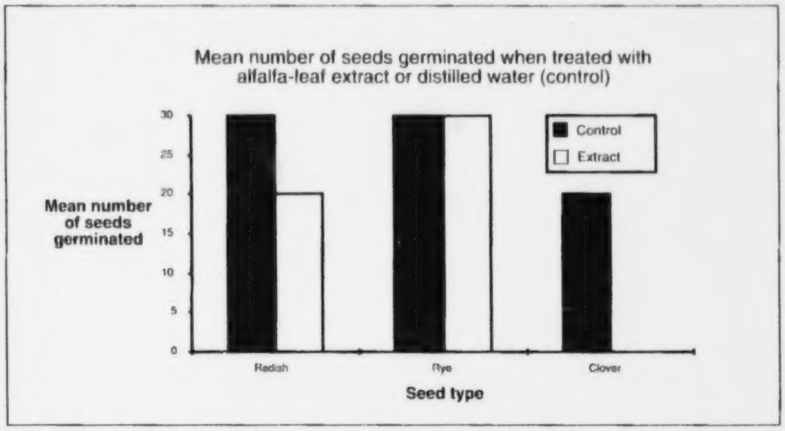
The results do not support the hypothesis. There is no difference between the experimental and the control groups of any of the target species. This suggests the alfalfa-leaf extract had no allelopathic effect.

Alternative Interpretation

The lack of negative effects does not rule out necessarily the possibility of allelopathy; it only indicates that there is no evidence of it. The allelopathic agent may not be water soluble or the extract may not have been strong enough to cause an effect.

Sample Data 4

The 3 target species may not respond equally to the alfalfa extract. Different species may have different sensitivities or different types of responses to the allelopathic substance. The germination of one species may be inhibited, but that of another may not be. See Graph C. Nitrogen-fixers such as clover may be affected indirectly through inhibition of the nitrogen-fixing bacteria in their roots.



Graph C. Effect of alfalfa-leaf extract on seed germination.

Interpretation

Radish and clover both showed reduced germination in the presence of alfalfa-leaf extract; clover showed a greater reduction in germination than did radish. Rye grass germination was not affected by alfalfa-leaf extract.

TEST QUESTION

Why might the alfalfa-leaf extract have different effects on the different species of plants?

Answer to Test Question

Some plants may be more resistant to the allelopathic substances produced by alfalfa. Others may not be sensitive to the substances at all.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After students have collected and analyzed the data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted their first experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- Does heat destroy the allelopathic agent?
- Could direct exposure to sunlight or ultraviolet light alter chemicals in plants that are known to produce allelopathic effects?
- Are there important allelochemicals in familiar fruits or vegetables?
- If bacteria grow in the leachate, what inhibits germination — the bacteria or the extract?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

- Allow students to bend a leaf of each plant to smell it for identification during the introduction to potted herbs.
- Use larger seeds. Radish, white clover, and rye grass seeds are too small to be handled effectively by most students who are visually impaired. Garden peas and some varieties of beans are adequate. Also, seeds that are used for edible sprouts, such as lentils, mung beans, and sunflower seeds, are good choices. (Park Seed, 1 Parkton Avenue, Greenwood, SC 29647 or Reay's Ranch Market, 3360 East Speedway, Tucson, AZ 85716).
- Do not use a magnifying glass. It is not an option for the blind and not satisfactory for students who are visually impaired.
- Alfalfa seeds present no problems as they are used in large quantities and not counted manually. Separate the alfalfa leaves from the roots by touch.
- Label beakers with braille. Labels can be made by inserting a sheet of self-adhesive labels into a braille slate.
- Reserve a section of a refrigerator shelf for students who are disabled. They should not handle other items to find their material labeled with braille.
- Use plastic margarine or cheese spread containers with lids, or resealable plastic bags in place of petri dishes. These containers measure about 5-cm high and make suitable sprouting chambers. Line them with filter paper or paper toweling cut to size. Petri dishes are too shallow to accommodate the larger seeds with their sprouts.
- Use a 10-mL disposable syringe to add water to the seeds. The barrel of the syringe can be notched to show the position at 10 mL. When shoots and roots appear, they should be measured with a braille ruler.

Deaf or Hard-of-Hearing

- Practice good communication skills. They are important not only for the student who is deaf or hard-of-hearing, but also for the teacher and classmates. The student who is deaf may be able to perform every portion of a biology experiment, but performance is no longer a solo act.
- Make lab partners aware that they must be alert to relay impromptu comments by the instructor during the investigation.
- Alert the lab group that they should be willing to inform their non-hearing

TEACHER'S NOTES

TEACHER'S NOTES

partner about dangers in the classroom unless the biology room is equipped with flashing lights, such as a fire alarm alert.

- Assign partners who have good note taking skills to deaf students who speak. Deaf students who neither read lips nor speak need skill with a note pad and friends who will assist them.

Gifted

- Explore variables that may affect allelochemicals such as concentration, time of year, or growing conditions of allelopathic plants or target species.

Manually Impaired

- Allow students with manual impairment to work with materials that they can handle most easily. For some it will mean using larger seeds such as sunflower seeds, peas, lentils, mung beans, squash, and green beans for the reaction with alfalfa solution. For others, it may mean taking notes with a tape recorder while the lab partners plant and evaluate seeds.
- Measure water in a short, wide graduated cylinder or a measuring cup if handling droppers requires finer motor skills than some students possess.

Mobility Impaired

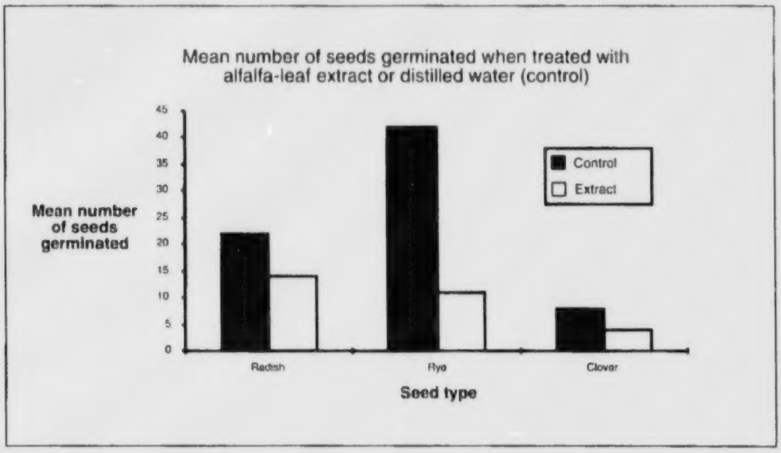
Mobility impaired students should have no problems with the experiments in this unit.

- Provide a table of suitable height, preferably one large enough for the lab group. Students in wheelchairs need a comfortable place to work. Where counter tops in the biology lab are too high to be reached from a seated position, or the student is unable to transfer to a chair, a portion of the apron of the table may need to be altered to allow the arms of the wheelchair to fit beneath it.
- Provide adequate space for wheelchairs to access supply areas within the classroom. If the room does not have adequate space, lab partners can provide these items.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment may also include the following:

1. The seeds of three plant species were exposed during germination to either distilled water or alfalfa-leaf extract that has allelopathic effects on certain plant species. See Graph D.



Graph D. Effects of alfalfa-leaf extract on seed germination of three plant species.

When treated with alfalfa-leaf extract, the clover seeds showed a lower germination rate than the radish and rye grass seeds. This was due to:

A. The effects of alfalfa-leaf extract.
B. An intrinsic characteristic of the clover.
C. The allelopathic effects of the alfalfa-leaf extract and factors that could be intrinsic to the clover species.
D. The radish and rye grass seeds not being sensitive to the allelopathic chemicals produced by the alfalfa.

2. An equal number of seeds were watered with distilled water and alfalfa-leaf extract to:

A. Show that the added nutrients in the leaf extract would help germination.
B. Compare the numbers of seeds that germinated when treated with distilled water versus those treated with alfalfa-leaf extract.
C. Test the distilled water for allelopathic chemicals.
D. Provide the amount of water the seeds needed to germinate.

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POSSIBLE SOURCES OF MENTORS

Check with biology and agriculture departments at local universities. Much research on allelopathy is done by agricultural researchers. A state, local, or federal agency might be able to refer you to possible mentors.

VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Teacher information for each variation is the same as that found in the Core Experiment, except as noted. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

Answers to Additional Test Questions

1. C
2. B

Answers to Questions and Analysis on Student Page

1. Answers will vary depending on the outcome of the experiment. However, previous experiments indicate that alfalfa-leaf extract appears to reduce germination in white clover and radish, but not in rye grass. Leaf growth is inhibited in all 3 species. Sensitivity varies among the species; white clover is the most sensitive.
2. Examples include inaccurate measurement of extract, distilled water, or seedling length; spacing of seeds; and poor germination conditions, such as an environment that is too cold.
3. Answers will vary.
4. See variations for suggestions.
5. Allelopathy is a type of interference competition. Competition between two species occurs when a resource, such as nutrients, is in limited supply. Interference competition occurs when one species directly affects the other, for example, by attacking it. Allelopathy is a form of interference competition that occurs among plants. Molecules produced by one plant, mostly secondary metabolites, escape into the environment and then affect the growth and development of neighboring plants.
6. Allelopathic plants may explain why some crops cannot be grown together and why a border separation between fields or crop ro-tation may be necessary. Allelochemicals have potential applications as "natural herbicides."

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TEACHER'S NOTES

VARIATION 1

The Effect of Different Concentrations of an Allelopathic Agent on Seed Germination and Growth

SYNOPSIS

Students will test the effects of different concentrations of the allelopathic agent produced by alfalfa leaves. They will determine if the concentration affects the germination and growth of seeds of the target species.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 15 g freshly cut alfalfa leaves
- 7 10-mL flasks
- 1 10-mL graduated cylinder
- 12 disposable 10 x 150-mm petri dishes
- 36 8-cm pieces of Whatman filter paper
- 7 disposable 5-mL plastic pipettes or syringes without needles
- 120 target plant seeds of the same species

HYPOTHESIS GENERATION

Question

Will the concentration of an allelopathic chemical influence its effects on other plants?

Sample Hypothesis

An increase in the concentration of the allelopathic agent will cause a decrease in the number of germinated seeds and in seedling growth.

Rationale

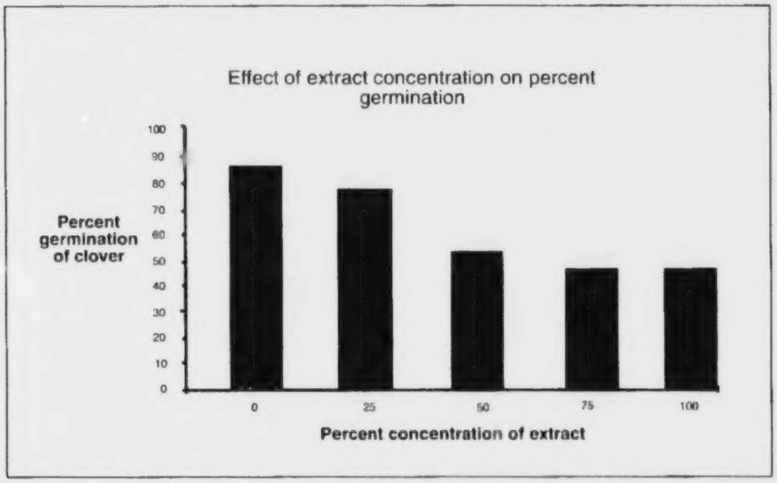
The effect of the allelopathic agent may depend not just on its presence, but on the quantity to which the target species is exposed.

Sample Experimental Procedure

1. Prepare 200 mL of highly concentrated alfalfa-leaf extract solution according to the procedure in the Core Experiment. To concentrate the solution, increase the amount of alfalfa leaves used to prepare the extract.
2. Prepare dilutions of 100%, 50%, 25%, and 0% using the concentrated alfalfa-leaf extract and distilled water. For the 100% solution, use the undiluted extract only. For the 75% solution, mix 7.5 mL extract with 2.5 mL distilled water; for the 50% solution, mix 5.0 mL of the extract with 5.0 mL of distilled water; for the 25% solution, mix 2.5 mL of the extract with 7.5 mL of distilled water; and for the 0% solution, use distilled water only.
3. Line each petri dish with 3 sheets of filter paper and label each dish with the appropriate concentration of the alfalfa-leaf extract solution. There should be 3 treatment dishes for each concentration.
4. Add 5.0 mL of distilled water to each control dish.
5. Add 5.0 mL of the appropriate concentration of leaf extract to each treatment dish.
6. Continue the experiment according to the sample procedure for the Core Experiment.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph E. Germination of seeds treated with increasing concentrations of alfalfa-leaf extract.

TEST QUESTION

Based on the results of your experiment, what happened to the number of germinated seeds as the concentration of alfalfa-leaf extract increased? Why do you think this occurred?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Use different sizes of syringes to measure the liquid to make the serial dilutions of the concentrated alfalfa extract. The barrels of the syringes should be marked appropriately. With many measurements to make in this experiment, the student may wish to use other methods.
- Weigh liquids on a balance in a container of known mass. With 1 mL weighed at 1 gram, serial dilutions can be made readily. Students who are blind readily use the double and triple beam balances. The weights on the beams slide into notches readily counted by touch to find the value. With a piece of tape placed at the zero point, the student is able to tell when the pointer is centered by touch. A quadruple beam is available in which the notch slide is calibrated with the braille ruler to show the number of grams per centimeter.
- Expect all students to present laboratory reports containing the same items. Students who use braille should write their report in braille for their records. For the benefit of the sighted teacher, a typewritten report should be presented.
- Use raised-line drawings if drawings are included. Labels should be in print and in braille. Braille graph paper is available or it can be made by the students on their brailler. When several bars are on the same graph (see Allelopathy Graph A), different tools and techniques are used to raise the area in each bar. A drawing such as Figure 2, which shows a beaker with leaves, stem, and roots in water, can be made as a raised-line drawing. A pointed tool sketches the drawing on braille paper that has been placed on a heavy rubber mat. (A clipboard with several layers of heavy cloth will serve also.) The size and type of lines are made by using

Interpretation

Accept the hypothesis if a decrease in the number of germinated seeds and the amount of growth of the seedlings was observed when there was an increase in the concentration of the allelopathic agent.

Reject the hypothesis if the number of germinated seeds and the amount of seedling growth was approximately the same or greater for all the concentrations of the alfalfa-leaf extract. The hypothesis also should be rejected if all the seeds exposed to the alfalfa extract showed less growth than the control seeds or if the degree of response is uncorrelated with concentration. This suggests that any amount of the allelopathic agent is harmful to seed germination and growth.

Answer to Test Question

Answers may vary depending on the outcome of the experiments. Generally, as concentration increases, the number of germinated seeds will decrease. This suggests that effects of allelopathic chemicals increase as the concentration increases.

a stylus, a dull pencil, a tracing wheel, a stick from an ice cream bar, or a special tool that leaves a dappled effect. Teachers and laboratory partners can likewise use this technique to make a quick drawing to explain some item in the laboratory.

- Provide students who are visually impaired the opportunity to examine models of plant parts and seeds if they are available in the science department.

TEACHING TIPS

- To facilitate the separation of the alfalfa roots from the soil, grow plants in a mixture of 4 parts sand to 1 part topsoil. Sift the topsoil before mixing to prevent large organic particles from adhering to the roots, or use potting soil.
- Students should keep the roots alive while collecting the extract. They can do this by gently washing off the sand and placing the plants in a small jar with enough distilled water to cover the roots. See Figure 5. Allow the plants to remain in the water for 2 to 4 days.

VARIATION 2

The Allelopathic Effects of Alfalfa Roots on Seed Germination and Growth

***Note to Teachers:** In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.*

SYNOPSIS

Students will test the effects of the extract of alfalfa roots on the germination and growth of radish, white clover, and rye grass seeds. They will determine whether the alfalfa roots produce allelopathic substances.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 1 tray of alfalfa, planted in sandy soil
- 1 50-mL beaker or baby food jar
- 4 kg (8 lbs) sand
- 1 kg (2 lbs) topsoil or potting soil

HYPOTHESIS GENERATION

Question

Do roots contain allelopathic substances?

Sample Hypothesis

Alfalfa roots produce allelopathic agents that reduce the number of germinated seeds and the amount of seedling growth.

Rationale

Students should provide their own rationale.

Sample Experimental Procedure

1. Prepare the alfalfa extract by allowing 5 g of alfalfa roots to steep in 25 mL of distilled water for 2 days. See Figure 5. Refrigerate after 1 day.

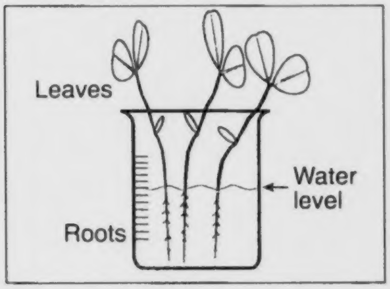
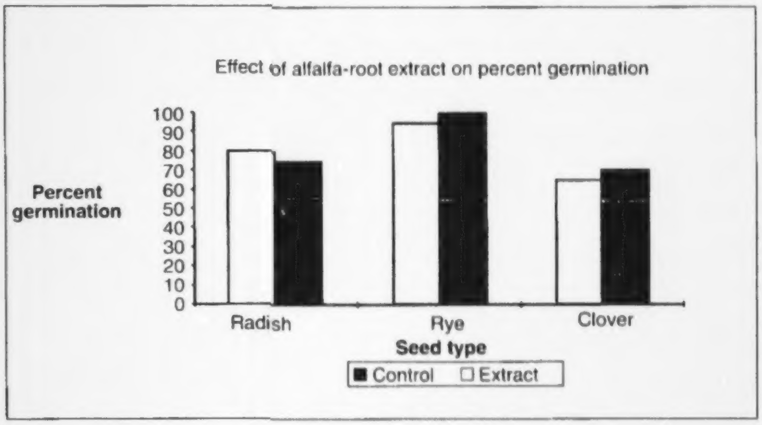


Figure 5. Roots of alfalfa sprouts submerged in water.

2. Continue by following the sample procedure for the Core Experiment using the alfalfa-root extract instead of the alfalfa-leaf extract.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph F. The effect of alfalfa-root extract on seed germination.

Interpretation

Accept the hypothesis that the roots of alfalfa plants produce an allelopathic substance if there is a decrease in the germination and growth of the seeds of radish, white clover, and rye grass.

Reject the hypothesis if the germination and growth of the seeds exposed to the alfalfa-root extract is the same as or greater than the control seeds.

TEST QUESTION

Did the alfalfa-root extract affect the seed germination or growth of any of the 3 target species?

Answer to Test Question

Answers will vary depending on students' results. However, tests generally show that alfalfa-root extract has no allelopathic effects on the germination of seeds.

VARIATION 3

The Allelopathic Effects of Alfalfa Seeds on the Germination and Growth of the Seeds of Other Plants

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will test the effects of the extract of alfalfa seeds on the germination and growth of radish, white clover, and rye grass seeds. They will determine whether the alfalfa seeds produce allelopathic substances.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 20 g alfalfa seeds
- 100 mL distilled water

HYPOTHESIS GENERATION

Question

Do alfalfa seeds produce allelopathic agents that reduce the germination and growth of seeds?

Sample Hypothesis

Alfalfa seeds produce allelopathic agents that reduce the germination and growth of radish, white clover, and rye grass seeds.

TEACHER'S NOTES

Interpretation

The hypothesis should be accepted if a decrease in the germination and growth of the seeds of radish, white clover, and rye grass was observed in the seeds treated with alfalfa-seed extract.

The hypothesis should be rejected if the radish, white clover, and rye grass seeds exposed to the alfalfa-seed extract showed the same amount or more germination and growth as the seeds grown with distilled water.

Answer to Test Question

Answers will vary depending on students' results. However, tests generally show that alfalfa-seed extracts have no allelopathic effects on the germination of seeds.

Rationale

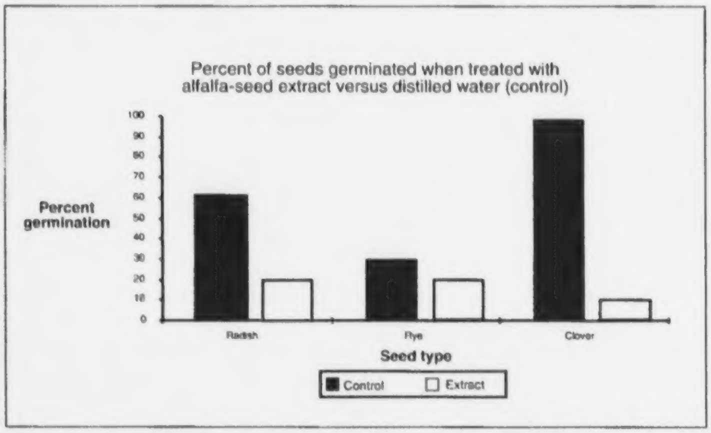
Students should provide their own rationale.

Sample Experimental Procedure

1. Make a seed extract using 20 g of alfalfa seeds soaked in 100 mL of distilled water for 2 days. Refrigerate after 1 day.
2. Continue by following the sample procedure for the Core Experiment using the seed extract instead of the leaf extract.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph G. Effects of alfalfa-seed extract on germination.

TEST QUESTION

Did the alfalfa-seed extract affect the germination or growth of any of the 3 target species' seeds?

VARIATION 4

A Comparison of the Allelopathic Effect of Leaf Extract from Different Plant Species on Radish, White Clover, and Rye Grass

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS

Students will test the allelopathic effects of various plants on the germination and growth of radish, white clover, and rye grass seeds. They will compare the allelopathic effects of these plants to the effect of alfalfa-leaf extract.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 250 g fresh cut leaves from celery, ragweed, mint, chickpea, walnut, or oak
- 250 g fresh alfalfa leaves
- 250 mL distilled water

SAFETY PROCEDURES

Ask students about allergies. Avoid plants that elicit student allergies.

HYPOTHESIS GENERATION

Question

Do the leaf extracts of any other plants affect the germination and growth of the target species?

Sample Hypothesis

Greater inhibition of germination and growth of seeds is obtained using black walnut-leaf extract as compared to alfalfa-leaf extract.

Rationale

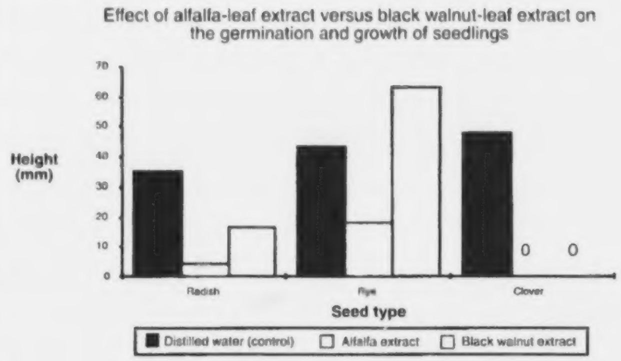
Black walnut is known to inhibit the growth of most plants. The black walnut extract differs from alfalfa extract and may affect more species or may affect some species more severely.

Sample Experimental Procedure

Duplicate the procedure for the Core Experiment, replacing the alfalfa leaves with leaves from the black walnut tree for the second treatment group. When preparing the extract, use 250 g of leaves in 250 mL of distilled water.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph H. Comparison of the effects of black walnut and alfalfa-leaf extracts on seeds.

TEST QUESTIONS

1. Compare the germination of clover seeds exposed to alfalfa-leaf extract to the germination of clover seeds exposed to black walnut-leaf extract. If there is a difference, what factors might explain it?
2. Why would different plants show varying degrees of allelopathy?

TEACHING TIPS

- If black walnut trees are not available in your community, test plants such as celery, ragweed, mint, chickpeas, walnut, and oak for allelopathy. Students may want to compare several species from the same genus.
- Results from the Core Experiment may be used while testing the other plant, but only if the tests are run concurrently.
- Black walnut is not common in New England.

Interpretation

Accept the hypothesis if the seeds showed less germination and growth when exposed to the black walnut extract than when exposed to the alfalfa extract or to distilled water. This suggests that black walnut leaves show a stronger allelopathic effect than alfalfa.

Reject the hypothesis if the same number or more seeds germinated and there was equal or greater growth when the seeds were exposed to the black walnut extract than when exposed to the alfalfa extract.

Answers to Test Questions

1. Answers will vary depending on student results. Differences may be due to different chemicals acting as the allelopathic agents.
2. Allelopathy helps plants compete with other plants for limited resources. Varying degrees of allelopathy in plants may suggest different levels of competitive stress or reflect intrinsic metabolic differences among plants.

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VARIATION 5

The Use of Allelopathic Plants as "Natural" Herbicides

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Some researchers are interested in whether allelochemicals can be used as natural herbicides against weeds, those plant species that are considered noncultivated or undesirable. Students will test the extract from black walnut leaves for allelopathic effects on seeds of a weed plant and a crop plant, such as sunflower.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 50 g black walnut leaves
- 60 weed plant seeds
- 60 sunflower seeds

HYPOTHESIS GENERATION

Question

Can black walnut-leaf extract be used as an herbicide?

Sample Hypothesis

An extract of black walnut leaves will prevent weed plant seeds from germinating but will not affect the germination of sunflowers.

Rationale

Black walnut is known to have an allelopathic effect on most plants, but is known to stimulate the growth of sunflower seeds. Therefore, it is reasonable to consider it as an herbicide for the weedy plants that may grow with sunflower crops.

Sample Experimental Procedure

Repeat the sample procedure for the Core Hypothesis. Prepare an extract from the leaves of a black walnut tree. Test for the effects of this extract on the germination of weed plant seeds and sunflower seeds.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

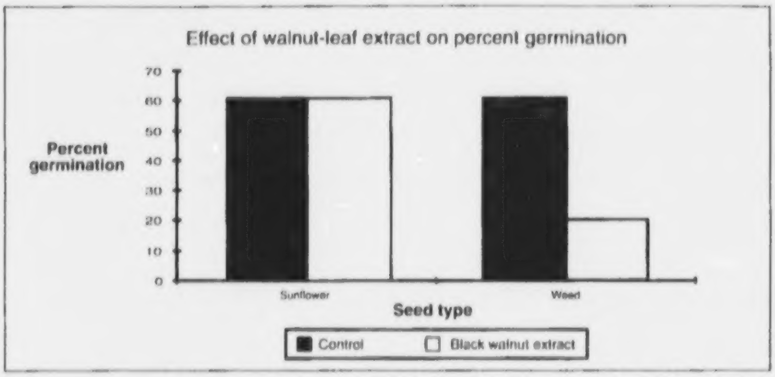
Possible results for this variation include:

- Germination rate varies.
- Germination rate is reduced.
- Neither the sunflower nor the weed plant seeds germinate.
- The sunflower seeds do not germinate, but the weed plant seeds do germinate.
- The sunflower seeds germinate, but the weed plant seeds do not germinate.
- Both the sunflower and weed plant seeds germinate.

The walnut-leaf extract can be considered an effective herbicide only if the sunflower seeds germinate, but the weed plant seeds do not.

TEACHING TIP

Students can test a variety of suspected allelopathic plants for their effects on several different common weeds and agricultural plants. They should try to find an allelopathic plant that prevents the growth of the weeds but does not harm the crop plants.



Graph 1. The effects of walnut-leaf extract on seed germination.

TEST QUESTION

Did you see any effect of the allelopathic plant against the weed seeds or the crop plant seeds? Based on these findings, would you recommend the allelopathic plant leaves as a natural herbicide?

VARIATION 6

The Effects of Light on the Production of Allelopathic Substances

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will test the allelopathic effect of the extract from alfalfa plants grown in different amounts of light. They will determine if the amount of light has an effect on the amount of allelopathic substance that the plant produces.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 2 4-L (1-gallon) pots
- 8 kg (16 lbs) topsoil or potting soil
- 50 g alfalfa seeds
- 3 30-cm^2 layers of 12-gauge cheesecloth
- 1 light meter

HYPOTHESIS GENERATION

Question

Will the amount of light that alfalfa seeds are grown in affect their allelochemical activity?

Sample Hypothesis

The leaf extract from alfalfa plants grown in less light will have diminished allelopathic effect on the germination and growth of radish, white clover, and rye grass seeds.

Interpretation

Accept the hypothesis if there is no difference in the germination rate of the sunflower seeds in the treatment group and the control group, but the weed plant seeds treated with the extract show less germination than those watered with distilled water.

Reject the hypothesis if the extract has an allelopathic effect on the sunflower seeds or if it did not have an allelopathic effect on the weed plant seeds.

Answer to Test Question

Answers will vary depending on the students' results.

TEACHING TIPS

- If a light meter from a camera is available, use it to determine the number of layers of cheesecloth needed to achieve the different levels of shading.
- Gauge of cheesecloth varies.
- Three layers of 12-gauge cheesecloth will reduce the light level by approximately 50 percent.

Rationale

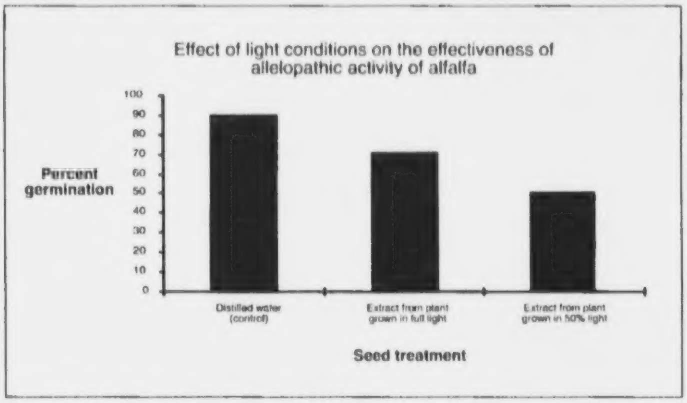
The amount of light from photosynthesis is known to have an effect on plant growth. Therefore, it may affect the production of allelopathic chemicals in alfalfa.

Sample Experimental Procedure

1. Grow 1 pot of alfalfa under 3 layers of cheesecloth or enough to reduce the light level by approximately 50 percent.
2. Grow the control alfalfa in full sunlight.
3. Prepare extracts from each set of alfalfa plants and treat the radish, white clover, and rye grass seeds as in the Core Experiment. Include a control of distilled water.
4. Compare the effects of the extract from plants grown in low light and plants grown in full light.

SAMPLE DATA ANALYSIS AND INTERPRETATION

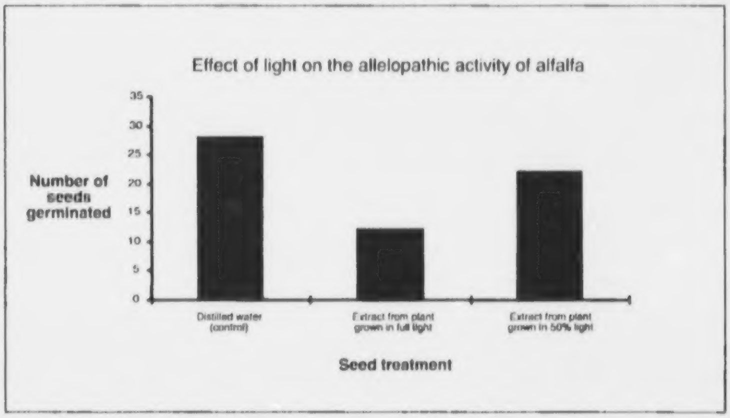
Sample Data



Graph J. Effects of the amount of growing light on the allelopathic activity of alfalfa.

TEST QUESTION

Based on the following graph, what would you conclude about the effects of light level on the production of allelopathic chemicals?



Graph K. The effect of light level on production of allelopathic chemicals.

Interpretation

Accept the hypothesis if the extract from the alfalfa plants grown in less light had a weaker allelopathic effect on the radish, white clover, and rye grass seeds compared to alfalfa seeds grown in full light.

Reject the hypothesis if the alfalfa plants grown with less light had an equal or stronger allelopathic effect.

Answer to Test Question

Based on the results shown in the graph, one would conclude that the extract from plants grown under decreased light did not have as strong an allelopathic effect as the extract from plants grown under full light. Note: the actual data students obtain may not agree with the results shown in the graph.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Use a light probe to determine the number of layers of cheesecloth needed to achieve the different levels of shading. The light probe has the same purpose and basic structure as the camera's light meter, but the light probe uses sound to register the amount of light. The higher the pitch, the greater the amount of light. Earphones are available to use with the light probe when the tone is disturbing to classmates.

VARIATION 7

The Effects of Nutrients on the Production of Allelopathic Substances

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students will test the allelopathic effects of the extract from fertilized and unfertilized alfalfa plants to determine if treatment with fertilizer has an effect on the production of allelopathic substances.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 2 4-L (1-gallon) pots
- 2 kg (4 lbs) topsoil
- 8 kg (16 lbs) sand
- 50 g alfalfa seeds
- all-purpose 20-20-20 liquid plant fertilizer
- target species seeds, such as white clover

SAFETY PROCEDURES

Follow the instructions carefully when preparing the fertilizer solution.
Wash hands before and after lab.
Do not eat or drink fertilizer solution.

HYPOTHESIS GENERATION

Question

Will fertilizer affect the production of allelopathic chemicals in alfalfa?

Sample Hypothesis

Treatment with a fertilizer will increase the allelopathic effect of alfalfa on white clover or another target species' seeds.

Rationale

As the availability of nutrients increases, more nutrients will be available for the production of allelochemical compounds.

Sample Experimental Procedure

1. Grow 2 pots of alfalfa in sandy soil. See Teaching Tips for sandy soil composition.
2. Treat the plants in 1 pot with fertilizer that can be applied as a liquid. Treat the control plants in the other pot with an equal amount of tap water, but no fertilizer.

TEACHER'S NOTES

TEACHING TIPS

- Use a mixture of 4 parts sand to 1 part topsoil.
- Sandy soil is lower in nutrients.
- 20-20-20 refers, respectively, to the nitrogen, phosphorous, and potassium levels in the fertilizer.

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TEACHER'S NOTES

Interpretation

Accept the hypothesis if the alfalfa plants that were treated with fertilizer had a greater allelopathic effect on the seeds. This suggests that the nutrient level of the growing medium affects the production of allelopathic substances.

Reject the hypothesis if the alfalfa plants grown with fertilizer did not have a greater allelopathic effect on the seeds.

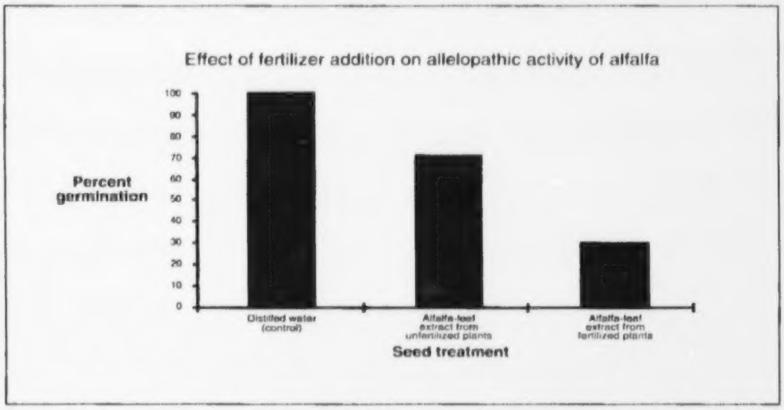
Answer to Test Question

Based on the results shown in the graph, the plants grown with low nutrient levels did not show as great an allelopathic effect as the plants grown with high nutrient levels. Note: the actual data students obtained may not agree with the results shown in the graph.

2. Continue the experiment by growing the seeds as in the Core Experiment. Germinate 1 set of seeds using extract from the fertilized alfalfa plants and 1 set using extract from the unfertilized alfalfa plants. Include a control germinated with distilled water.

SAMPLE DATA ANALYSIS AND INTERPRETATION

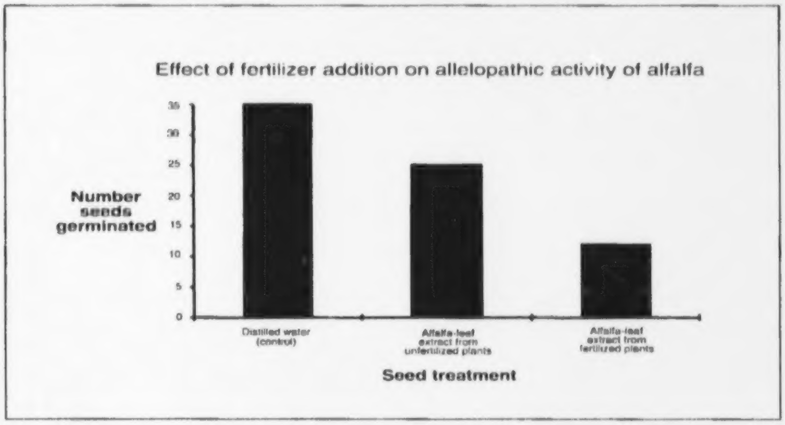
Sample Data



Graph L. Effect of fertilizer on alfalfa's allelopathic activity on a target species.

TEST QUESTION

According to the following graph, what would you conclude about the effects of nutrient level on the production of allelopathic substances?



Graph M. Effect of fertilizer on alfalfa's allelopathic activity on a target species.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Use 20 drops/mL of liquid fertilizer to provide a specific number of drops per liter. A bottle of diluted fertilizer should be provided for students who are blind.
- Use graduated cylinders that have raised lines on the outside that can be counted by running a fingernail or a plastic card down the side of the cylinder.
- Detect the meniscus in clear water with a light sensor. Liquid with a slight coloring is easier to read.

VARIATION 8

The Effect of Herbivory on the Production of Allelopathic Agents

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will test the allelopathic effect of alfalfa plants that have been subjected to herbivory, or the grazing by insects or other organisms, as compared to the effect of plants that have been protected from herbivory. Students will determine whether herbivory affects the production of allelopathic agents.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 2 4-L (1-gallon) pots
- 8 kg (16 lbs) topsoil or potting soil
- 1 pair of scissors

HYPOTHESIS GENERATION

Question

Will the allelopathic activity of alfalfa be affected by herbivory or grazing?

Sample Hypothesis

The extract from alfalfa plants that have been exposed to herbivory will have a greater allelopathic effect on seed germination.

Rationale

It is known that plants can produce protective chemical substances in response to herbivory. These substances also may have observable allelopathic effects.

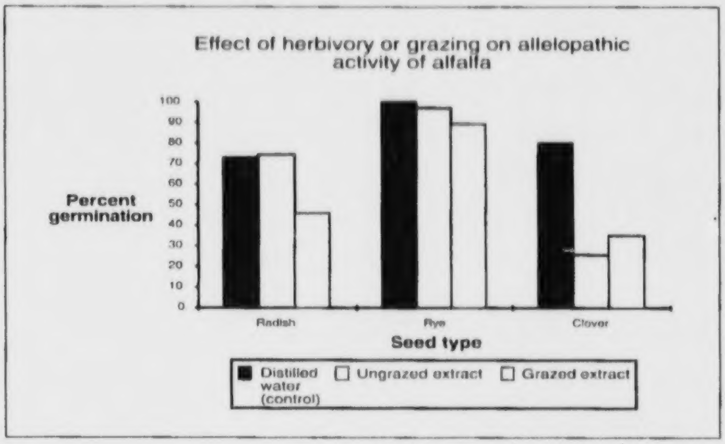
Sample Experimental Procedure

1. Plant 2 pots of alfalfa.
2. After about 7 days, trim approximately 1 cm off the tops of the alfalfa plants in 1 of the 2 pots to simulate the effects of herbivory.
3. Grow the control pot of plants alongside the treatment pot, but do not trim the control plants.
4. Continue to grow the plants for an additional 7 days.
5. Harvest the alfalfa plants from each pot after 14 days of growth and repeat the procedure used in the Core Experiment with each pot of alfalfa.

TEACHER'S NOTES

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph N. Comparison of the effects of alfalfa extract obtained from plants that have been subjected to herbivory.

TEST QUESTION

Based on your experiments, does herbivory affect the production of allelopathic agents in alfalfa? How do your data support your answer?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Students who are visually impaired should have no difficulty in performing all parts of this experiment on the effect of herbivory on the production of allelopathic agents. Provide an assigned area to locate plants labeled in braille.

VARIATION 9

The Influence of Plant Age on the Allelopathic Effect of Alfalfa

***Note to Teachers:** In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.*

SYNOPSIS

Students will determine whether the allelopathic effect of alfalfa plants varies with the age of the plant.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 4 4-L (1-gallon) pots
- 8 kg (16 lbs) topsoil or potting soil
- 100 g alfalfa seeds
- 1 artificial light bank
- 1 24-hour timer

Interpretation

The hypothesis should be accepted if the extract from the plants that were subjected to herbivory had a greater allelopathic effect on the seeds than extract from ungrazed plants. This would suggest that herbivory influences the production of allelopathic substances.

The hypothesis should be rejected if the extract from the plant exposed to herbivory does not have a greater allelopathic effect on the seeds than extract from the ungrazed plant. This would suggest that herbivory does not influence the production of allelopathic substances.

Answer to Test Question

Answers will vary depending on experimental results.

TEACHING TIPS

- Factors, such as day length, may vary during the 8-week growing period. So, it is best to grow the alfalfa under artificial lights with timers. Plants should receive 16 hours of light and 8 hours of dark.
- A modification of this variation is to examine the effects of plant age on the allelopathic agents produced by trees. Local park authorities may be able to tell you the age of the trees. If you cannot determine the exact age of the tree, you can estimate a range of "younger" to "older" trees based on trunk diameter.

HYPOTHESIS GENERATION

Question

How does the age of a plant affect the potency of allelopathic chemicals?

Sample Hypothesis

The leaf extract from older alfalfa plants will have a greater effect on the germination and growth of seeds.

Rationale

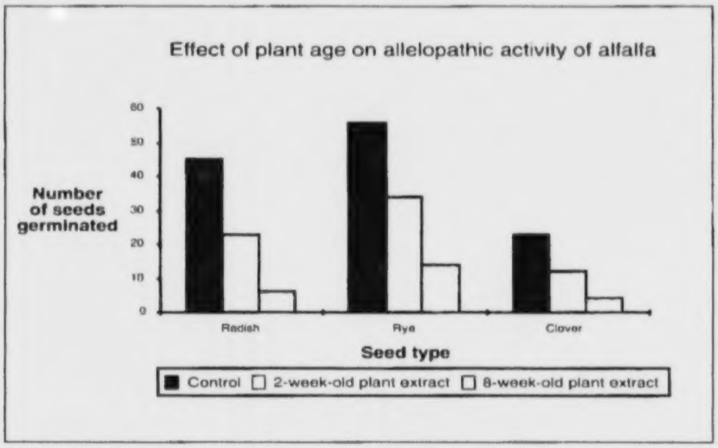
Concentration of allelochemicals can vary with the age of the plant. Therefore, the intensity of the allelopathic effect may vary with the age of the plant.

Sample Experimental Procedure

1. Plant 1 pot of alfalfa.
2. Six weeks later, plant the second pot of alfalfa.
3. Grow plants 2 more weeks.
4. Repeat the Core Experiment to test the allelopathic effects of plant age.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph O. Effects of plant age on the allelopathic potency of alfalfa-leaf extract.

TEST QUESTION

Did the effects of the allelopathic substance on seed germination and growth vary with the age of the alfalfa plant?

Interpretation

Accept the hypothesis if the older alfalfa plants had a greater allelopathic effect on the germination and growth of the seeds.

Reject the hypothesis if there was no difference in the allelopathic effect of the alfalfa extract from plants of different ages or if older plants had a lesser effect than younger plants.

Answer to Test Question

Answers will vary depending on students' experimental results.

TEACHING TIPS

- Agricultural and garden supply houses usually have a good supply of inoculum for nitrogen fixation.
- To facilitate the separation of the alfalfa roots from the soil, prepare a mixture of 4 parts sand to 1 part topsoil to grow the alfalfa. Sift the topsoil before mixing or use potting soil to prevent large organic particles from adhering to the roots.
- Sterilize the soil in an autoclave or pressure cooker at 15 pounds per square inch (psi) for at least 15 minutes. This will kill all soil bacteria. Keep the soil wrapped in several layers of heavy duty aluminum foil to prevent contamination until it is needed.
- Plants can be dried in envelopes or in any type of folded paper.
- Plants also can be dried in a conventional oven set at 200°F or air dried.

VARIATION 10

The Effect of Allelopathic Substances on the Formation of Root Nodules

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.

SYNOPSIS

Students will determine if the extract from alfalfa leaves will affect the formation of root nodules in white clover by symbiotic, nitrogen-fixing bacteria.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 120 white clover seeds, or another plant known to nodulate
- 2 kg (4 lbs) sterile, sandy soil
- 1 autoclave or pressure cooker
- 0.3 g inoculum
- 12 10-cm diameter plastic pots
- 1 microscope
- 1 drying oven or conventional oven (*optional*)

SAFETY PROCEDURES

Students should wash hands well after handling the inoculum.

The teacher should supervise all autoclaving of the soil. The steam is extremely hot, and sandy soil takes 30 to 60 minutes to cool for handling.

HYPOTHESIS GENERATION

Question

Is the formation of root nodules of legumes affected by alfalfa-leaf extract?

Sample Hypothesis

The extract from alfalfa leaves will inhibit the formation of root nodules by symbiotic, nitrogen-fixing bacteria.

Rationale

The chemical interaction between the legume and the nitrogen-fixing bacteria is affected negatively by allelochemicals.

Sample Experimental Procedure

1. Prepare alfalfa-leaf extract as described in the Core Experiment.
2. Fill 12 pots with sterilized sandy soil 1 cm from the top.
3. Label 3 pots with each of the following treatments:
   - no inoculum/treated with distilled water
   - no inoculum/treated with alfalfa-leaf extract
   - inoculum/treated with distilled water
   - inoculum/treated with alfalfa-leaf extract

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4. Plant 10 clover seeds in each pot. Water each pot with either distilled water or the alfalfa-leaf extract as the pot's label indicates.
5. Separate the pots to be inoculated from those that will not be inoculated.
6. Inoculate the appropriate pots by sprinkling 0.1 g of inoculum onto the soil. Be sure to inoculate well away from the uninoculated soil so it does not become contaminated.
7. Grow the clover for 10 to 14 days.
8. Gently remove the plants from their pots with roots and soil intact.
9. Carefully separate 5 plants from the soil and rinse them to remove any residual soil.
10. Use a microscope to count the number of nodules on each plant's roots. See Figure 6.

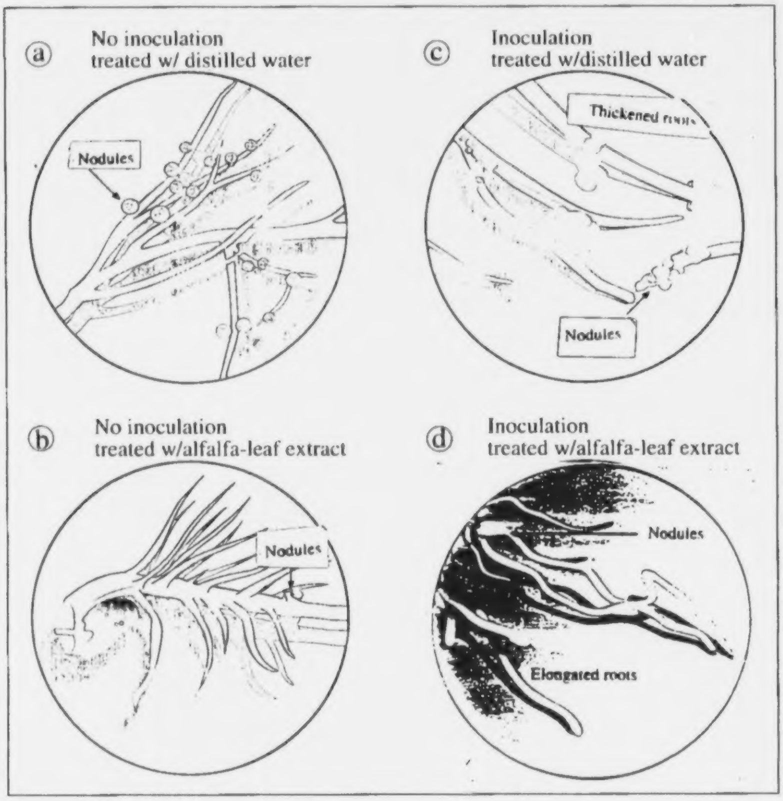
ⓐ No inoculation treated w/ distilled water
Nodules
ⓒ Inoculation treated w/distilled water
Thickened roots
Nodules
ⓑ No inoculation treated w/alfalfa-leaf extract
Nodules
ⓓ Inoculation treated w/alfalfa-leaf extract
Nodules
Elongated roots

Figure 6. Results of alfalfa-extract on white clover nodule development.

11. Harvest the remaining plants and rinse off the soil.
12. Dry the plants in a drying oven at 80°C. You may wish to separate roots from stems and leaves before drying in order to analyze them separately.
13. Once plants are dry, determine the biomass (dry mass).

TEACHER'S NOTES

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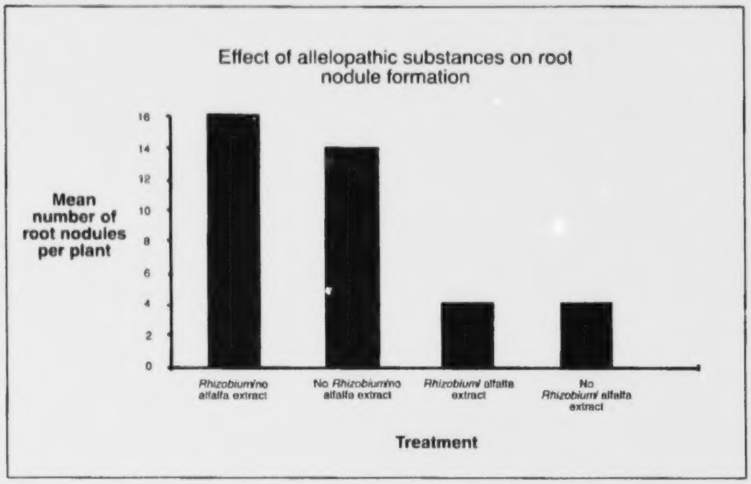
SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph P. Effect of alfalfa-leaf extract on the number of root nodules.

TEST QUESTION

Based on the results above, describe the effects of cultivating alfalfa and clover in the same section of land for a period of 10 years.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have students assist in planting and caring for the alfalfa plants. They should not handle the inoculum unless they are wearing rubber gloves.
- Wash the root nodules free of the inoculum for student examination.
- Have laboratory partners inform visually impaired students of the results of the microscopic work.

Manually Impaired

- Where there are investigations such as Variation 10 that use nitrogenous inoculum, it is best that students with manual impairments watch their lab partners. Some might be able to put seeds in the flower pots but would need friends to carry large items to the growing area. They need lab partners who not only will aid them but who will see that they also share the work when possible.

Interpretation

Accept the hypothesis if fewer nodules were formed by the clover plants that were treated with the alfalfa-leaf extract.

Reject the hypothesis if there was no difference in the number of nodules formed by the clover plants that were treated with alfalfa extract and the plants treated with distilled water.

Answer to Test Question

The alfalfa and clover will grow well the first 2 or 3 years. Eventually, the alfalfa may become chlorotic and will not thrive. If fertilizers containing nitrogen are added to the soil, the alfalfa crops will thrive. After this time, the clover will fail to thrive.

A Study of Allelopathy in Plants

Directions for Students

INTRODUCTION

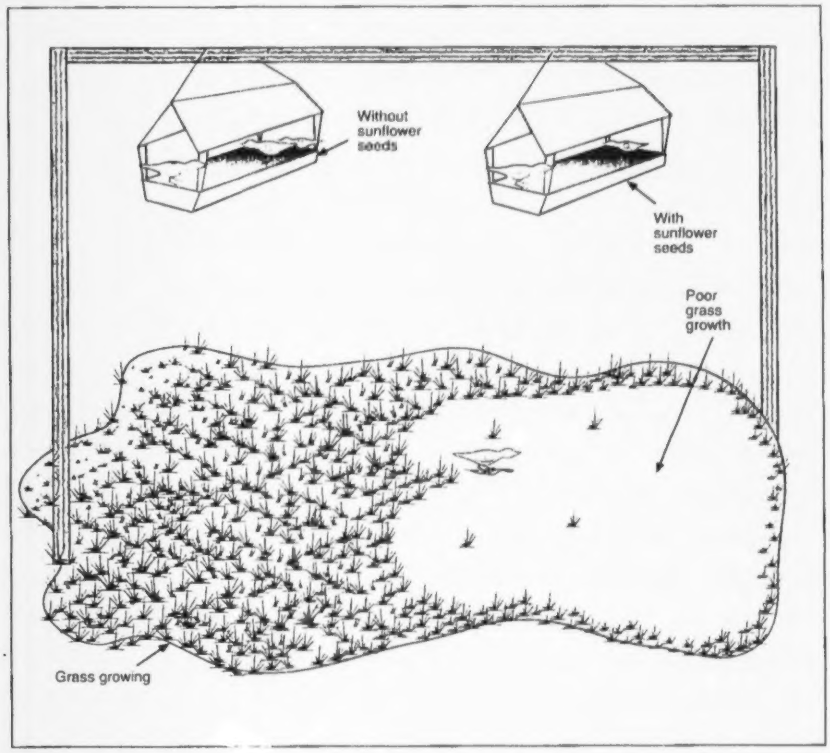


Figure 1. Two bird feeders located in the same area. One has sunflower seeds; the other does not.

Have you ever noticed bare spots under certain trees where flowers, even weeds (uncultivated plants), will not grow? Look at Figure 1. What observations can you make? Have you observed this in your community? In 300 BC, Theophrastus realized that planting chick peas made the soil unsuitable for many other plants. Earlier records of allelopathy date back before 300 BC when Democritus reported that weeds could be controlled by using naturally occurring plant products and that trees could be killed by treating their roots with a mixture of lupine flowers soaked in hemlock juice. In 1 AD, Pliny, the Roman naturalist, reported that barley and bitter vetch prevented the growth of other plants in the same soil. Later reports showed that even the rain or dew washing from pine needles onto crop plants caused damage.

In more recent history, A. B. Massey (1925) determined that toxic substances released into the soil by walnut trees inhibited the growth of almost all other plants. It eventually was suggested that some plants exude chemical substances that are injurious to other plants. This phenomenon has been observed in nature, most notably in California's Mojave Desert where creosote shrubs exclude burro-weed by exuding a chemical from their roots that may inhibit the root growth of the burro-weed (Dutton, 1993). Some researchers, however, contend that other factors, such as competition for light or nutrients, are the reason why certain plants are unable to grow together and that allelopathy is not a factor. How might you test whether a plant has allelopathic effects on another plant? With your class, you will develop a hypothesis to answer a question about allelopathy and design an experiment to test this hypothesis. This experiment will serve as the basis of other experiments.

OBJECTIVES

At the end of this lab, you should be able to:

- Identify the parts of a plant that may produce allelopathic substances.
- Describe the observable effects of allelopathy on seed germination, seedling appearance, and growth of roots and leaves.
- Discuss the ecological implications of allelopathy.

SAFETY NOTES

Let your teacher know if you are allergic to any of the plants used in this experiment.

Wash your hands after touching plants.

Students with allergies should wear nonallergenic gloves.

MATERIALS NEEDED

For each group of four students, you will need the following:

- 5 g freshly cut alfalfa leaves
- 1 pair of scissors
- 1 150-mL glass beaker
- 18 disposable 10 x 150-mm petri dishes
- 1 permanent marking pen
- 54 sheets of 8-cm Whatman filter paper
- 60 seeds each of radish, white clover, and rye grass
- 1 50-mL flask
- 2 5-mL disposable plastic pipettes or syringes without needles

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of allelopathy. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

Think about what effects an allelopathic plant might have on the germinating seedlings of another species. From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis.

Answer the following questions:

1. What is the question you are investigating?
2. Why is it important to control variables other than the one you are investigating?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin the experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. Do these data support your hypothesis? Discuss the evidence you used to come to this conclusion.
2. What were some of the major sources of error in your experiment?
3. What changes, if any, would you make in your procedures?
4. What other experiments would you do as an extension of this study?
5. How does this lab relate to ecological principles in nature?
6. How might some of your findings be applied, for example in agriculture?

DESIGN OF VARIATIONS OF CORE EXPERIMENT

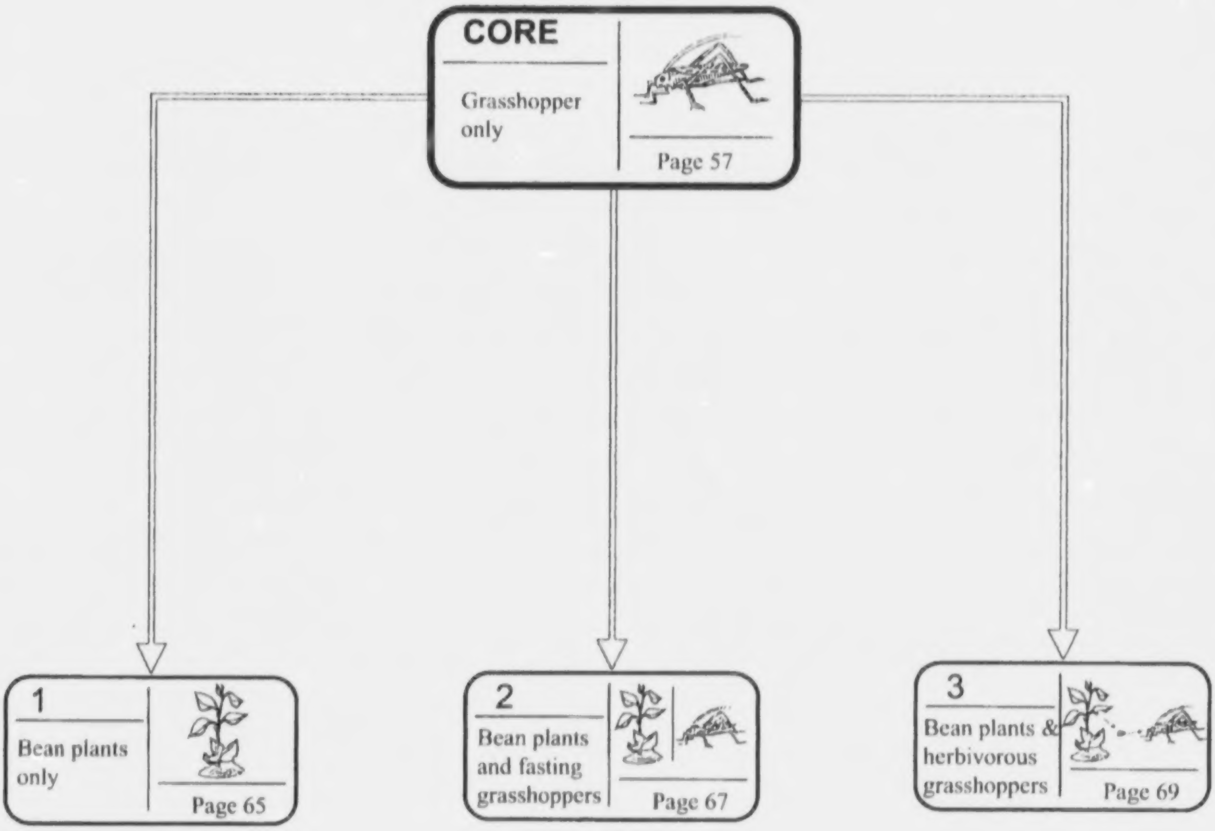
After you have discussed the results of the Core Experiment with your classmates, write down questions that occurred to you as you tested the allelopathic effects of alfalfa on seed germination. Design an experiment that is quantifiable and write your procedure in a numbered list of steps. Questions other students have studied include the following:

- Will the concentration of an allelopathic chemical influence its effects?
- Do roots contain allelopathic substances?
- Do alfalfa seeds produce allelopathic agents that reduce the germination and growth of seeds?
- Do the leaf extracts of any other plants affect the germination and growth of the target species?
- Can black walnut be used as an herbicide?
- Will the amount of light that alfalfa seeds are grown in affect their allelochemical activity?
- Will fertilizer affect the production of allelopathic chemicals in alfalfa?
- Will the allelopathic activity of alfalfa be affected by herbivory or grazing?
- How does the age of a plant affect the potency of allelopathic chemicals?
- Is the formation of root nodules of legumes affected by alfalfa-leaf extract?

NOTES

Biosphere 3 — It's a Small World After All

At-A-Glance Map of the Core Experiment and Its Variations


CORE
Grasshopper only
Page 57
1
Bean plants only
Page 65
2
Bean plants and fasting grasshoppers
Page 67
3
Bean plants & herbivorous grasshoppers
Page 69

NOTES

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Biosphere 3—It's a Small World After All

SYNOPSIS FOR CORE EXPERIMENT

Students will investigate and analyze mathematically the interactions that take place among several variables of a closed ecosystem.

APPROPRIATE BIOLOGY LEVEL

Introductory, intermediate, and advanced

TEACHER PARTNERS

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Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 65. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Develop a simple mathematical model that investigates the complicated processes and systems of an artificial ecosystem.
- Describe the role each biotic and abiotic variable plays within the ecosystem.
- Observe, record, and interpret how different components of the ecosystem change as they interact with each other and the environment.
- Discuss how models are used to investigate complicated processes and systems.
- Discuss the difficulties of controlling all variables in a complicated system.

MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 4 sheets of graph paper
- 1 calculator
- 1 number cube or die
- 1 or more class "closed bottle" systems (*optional*)

SAFETY PROCEDURES

None to consider.

DIRECTIONS FOR SETTING UP THE LAB

One day before

- Collect materials for the Core Experiment.

(continued on p. 56)

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Discuss the use of models and how scientists use mathematical models to study living systems.
- Discuss how to develop a simple mathematical equation to study Biosphere 3.

Day 2 (45 minutes)

- Implement the mathematical equation.
- Collect, analyze, and discuss these data.

Day 3 (45 minutes)

- Design a model based on the Core Experiment of another variable.

PREPARATION TIME REQUIRED

15 minutes

- Gather materials.

3 hours

- Gather materials and organisms and set up the class "closed bottle" for observation (*optional*).

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TEACHER'S NOTES

(continued from p. 55)

One month before (optional)

- Set up a classroom aquatic "closed bottle" or biosphere in a large container, such as a 5-gallon drinking water bottle. Include aquatic organisms such as snails and *Elodea* or other submerged aquatic vegetation (SAV).
- Have students collect mud and water from areas around their home community and seal in a bottle. Place these bottles in a classroom window or under a Gro-Lite®.

TEACHER BACKGROUND

Content Information

The concept of a biosphere is not a new one. In 1875, the Austrian geologist Eduard Suess introduced the term to describe "the 'envelope' around the planet [Earth] that was inhabited by life" (FAQ, 1998). Today, the term biosphere can be described as the many ecosystems of the Earth that interact with each other. An ecosystem is a community of organisms and all of its elements and their functions that exist in nature as an entity. From space, the Earth appears as a self-contained sphere of life and is often referred to as Biosphere 1.

The advent of the space age brought a new perspective of the biosphere—how to make an artificial "biosphere" for extended periods of time. With the focus on space travel, the Soviets began experiments on closed biological systems. In 1961, Soviet scientists sealed physician Evgenii Shepelev with a tank containing algae for his oxygen source inside a 160-cubic-foot cylinder. After 24 hours, he had to leave the system. Although the system had been successful at cycling oxygen and carbon dioxide, it was far too simple to accommodate all of the waste gases produced by himself and the algae (Closed systems, 1998).

In 1968 at the University of Hawaii, microbiologist Clair Folsome studied totally contained systems of microorganisms, rather than humans and higher plants. Folsome sealed samples of ocean water in flasks so nothing could enter or leave them and set them in indirect light on a lab windowsill. To his amazement, the community of microorganisms continued to live. In fact, some still survive today. Folsome continued to study these micro-ecosystems, expanded his collection with samples from other locations, and developed ways to evaluate the systems while maintaining their isolation. He discovered that if the microbial communities were diverse enough they could live indefinitely and establish their own ecosystems (Gentry, 1992). He had rediscovered an observation by the Russian geochemist, Vladimir Vernadsky, that "as life evolves, it actually changes the environment in which it evolves" (Sieloff, 1995).

By 1972, the research started by Shepelev had advanced considerably to the creation of the Soviet Bios-3. This enclosed system was much larger and housed several people and a hydroponic garden, but was still not totally self-contained. Wastes had to be exported and a protein source imported. The challenge remained to create a system that could be self-contained.

On September 26, 1991, this challenge was addressed with completion and sealing of Biosphere 2. It was constructed as a closed system to study how the Earth works. It contains five biomes that simulate those on Earth—ocean, rainforest, marsh, savanna, and desert—with over 4,000 species of organisms. Artificial communities include an agricultural area, micro city, and technosphere for human habitation (Sieloff, 1995).

In Biosphere 1 (Earth), the amount of temperature, rain, and light in any one area determine what kind and how much plant life will grow. Plants change the atmosphere by producing oxygen and using carbon dioxide found in our atmosphere. Organisms feed upon each other and interact with their non-living environment. Organisms depend upon energy from the sun locked in organic compounds that are stored in plants. Nutrients, water, oxygen, carbon, and nitrogen are constantly being recycled in nature. Change occurs when organisms modify their environment, both short term (night and day) as well as long term (succession). Throughout all these interactions, energy travels in a one-way path while all matter is cyclic. Despite population fluctuation, our biosphere maintains stability throughout time.

Organisms interact in mathematically measurable ways. Mathematical models predict the effects of changes in variables. Probability is an example of a common use of mathematical modeling in genetics. In this activity, students will model mathematically the effects of one and multiple variables upon the resources of a closed ecosystem—Biosphere 3. Isolating one variable from this complex system to study is a daunting challenge, as the mathematical modeling of this lab will reveal.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

Correct Concept	Misconception
• Both plant and animal cells respire.	• Only animals undergo cellular respiration.
• Ecosystems are very complicated systems that are difficult to analyze.	• Variables in a complicated system can be controlled.
• Plants and animals share many of the same physiological processes.	• Plants and animals have little in common.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

If this is the first time your students have designed an experiment, the following paper and pencil exercise will provide design practice.

Introduction—The Biosphere 3 Model

The closed bottle or biosphere is a traditional biology classroom activity that offers a wealth of opportunities for student observations throughout the year and discussion of the interrelationship of biotic and abiotic factors. Possible ways to introduce this activity include:

- Have students discuss what factors are necessary for the organisms in the class biosphere system to survive. Discuss Biosphere 2 and how what looks like a very simple system is actually quite complex.
- Introduce the concept of models and why they are used. Models that students may be familiar with are the stick and ball models used for chemical molecules. These are physical models. Have them list other physical models and how they are used. Models may be mathematical also. Such models can be used to predict global warming, forecast the weather, or analyze the spot patterns on leopards or stripe patterns on zebras. Mathematical models can be very useful in science, but the assumptions on which they are based must be considered carefully. For example in the orange roughy model, scientists predicted that the optimum age to harvest orange roughy for food was 40 years of age after they had reproduced. Unfortunately, the life span of the orange roughy was miscalculated. Orange roughy do not reproduce until age 80. Ask students why mathematical models need to be designed carefully.

TEACHING TIPS

- Modeling may be done on any of several levels depending upon student interest. Possible modeling approaches include:
  - graphing simple algebraic equations that assume constant rates of change.
  - generating a calculus-based equation to model variable rates of change among variables in the model.
  - using graphing calculators for calculations.
  - using a computer modeling program such as *Stella*®.
  - using spread sheet software to perform repetitive calculations.
  - writing a computer program.
- Using simulation software such as *SimIsle*™, *SimCity*™, or *BioBLAST*™.

TEACHER'S NOTES

- For this activity, have students imagine Biosphere 3 is an imaginary container that can be sealed after the introduction of bean plants and grasshoppers. The relationship and effects of these species' introduction into the imaginary container are analyzed mathematically. Each biosphere experiment begins by determining how many bean plants and/or how many grasshoppers will be introduced and by specifying the starting conditions within the biosphere. Provide students with the following assumptions of Biosphere 3 (Note to the teacher: In order to keep the mathematical model simple, the relationship between temperature, pressure, and volume within the biosphere is not considered.):

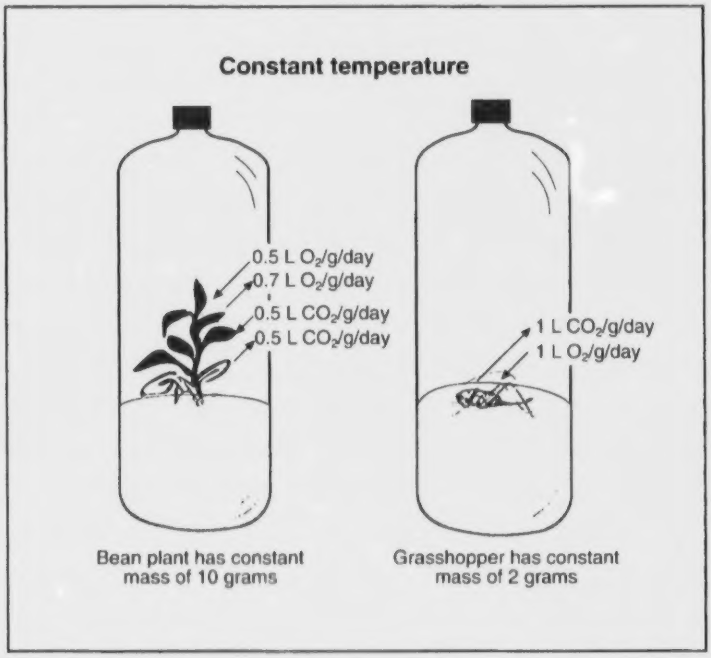


Figure 1. Biosphere 3.

1. Atmosphere contains 1 L O_2 and 1 L CO_2 at the beginning of the experiment.
2. Biosphere 3 can be inhabited by grasshoppers and/or bean plants only.
3. Each grasshopper that inhabits Biosphere 3:
   - has a mass of 2 g throughout the experiment.
   - uses 1 L O_2/gram body mass/day.
   - produces 1 L CO_2/gram body mass/day.
4. Each bean plant that inhabits Biosphere 3:
   - has a constant mass of 10 g.
   - uses 0.5 L O_2/gram plant mass/day.
   - produces 0.7 L O_2/gram plant mass/day.
   - uses 0.5 L CO_2/gram plant mass/day.
   - produces 0.5 L CO_2/gram plant mass/day.
5. All rates, such as the rate of oxygen consumption by grasshoppers, remain constant while in Biosphere 3.
6. Temperature remains constant and the sun shines 24 hours per day.
7. Grasshoppers do not need to eat or drink while they are in Biosphere 3.
8. Plants do not need water while in Biosphere 3.
9. Ignore the fact that the volume of the container does not change to accomodate an increase or decrease in the gas volume. This is done in order to keep the model simple.

Note: Part of the activity can include examining the above assumptions. For example, given that the students understand the basics of physiology:

- Is it reasonable to assume that plants will consume the same amount of CO_2 daily as they produce?
- Is it reasonable to assume that the rate of CO_2 production (on a per gram body weight basis) for a grasshopper is greater than that of a bean plant?

Explain that the classroom biosphere, due to its complex nature, lends itself very well to study by mathematical models.

For the Core Experiment, consider the following dialogue approach to develop student understanding of the process of equation development.

Question

Assume that there is 1 L O_2 in Biosphere 3 at the start of the experiment. How much O_2 is left after 1 hour?

Answer

The amount of O_2 remaining in Biosphere 3 after 1 hour is equal to the starting amount less the amount that is used in 1 hour. This is a "word equation." We could write it in mathematical form to look like this:

$$\begin{aligned} O_2 \text{ remaining} &= \text{Starting amount} - \text{Amount used} \\ &= 1\ \text{L}\ O_2 - X\ \text{L}\ O_2 \end{aligned}$$

Question

In our equation, the amount of O_2 used is an unknown (X). What determines how much O_2 will be used in Biosphere 3 and how fast it will be used?

Answer

The amount of O_2 used and the rate at which it is used is determined by the kinds of living things sealed inside Biosphere 3.

Assume that 1 grasshopper is the only organism inside Biosphere 3. In our experiment, a grasshopper has a mass of 2 grams and uses 1 L O_2 per gram of body mass per day. We can use this information to determine "X" in our equation above.

Question

How much O_2 will a 2-gram grasshopper use per day?

Answer

Since 1 grasshopper uses 1 L O_2 per gram of body mass per day, and our grasshopper has a mass of 2 grams, it will use 2 L O_2 per day.

Question

Can you write this answer as an equation in mathematical form?

Answer

$$\begin{aligned} O_2 \text{ used per day} &= \frac{1\ \text{L}\ O_2}{\text{gram}} \bullet \frac{2\ \text{grams}}{\text{grasshopper}} \bullet 1\ \text{grasshopper} \\ &= 2\ \text{L}\ O_2 \end{aligned}$$

Question

So, 1 grasshopper will use 2 L O_2 per day. How much will it use in 1 hour?

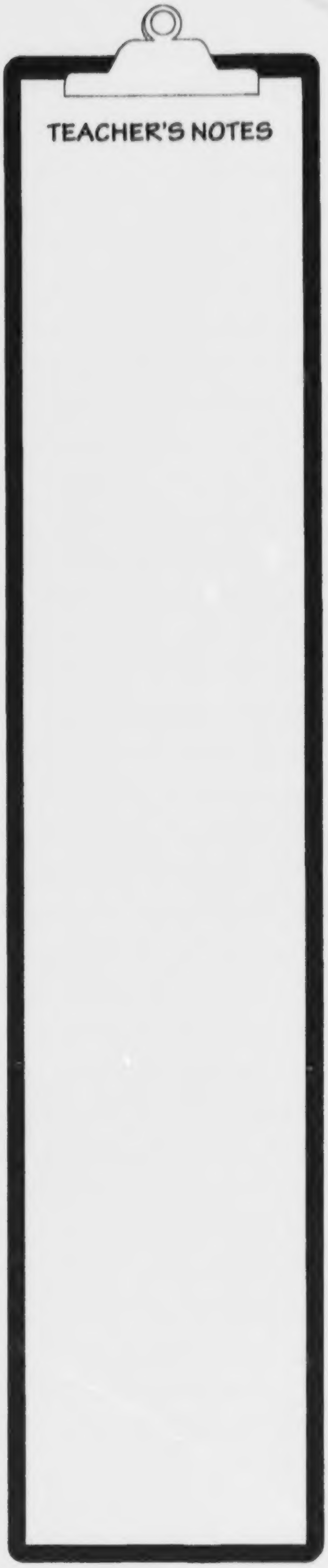
Answer

$$\begin{aligned} O_2 \text{ used per hour} &= \frac{2\ \text{L}\ O_2}{\text{day}} \bullet \frac{1\ \text{day}}{24\ \text{hours}} \bullet 1\ \text{hour} \\ &= 0.083\ \text{L} \end{aligned}$$

Question

Let's go back to the original question above. If Biosphere 3 starts out containing 1 L O_2 and 1 grasshopper, how much O_2 will it contain after 1 hour?

(continued on p. 60)



TEACHER'S NOTES

(continued from p. 59)

Answer

O_2 remaining $= 1 \text{ L } O_2 - 0.083 \text{ L } O_2$
$= 0.917 \text{ L } O_2$

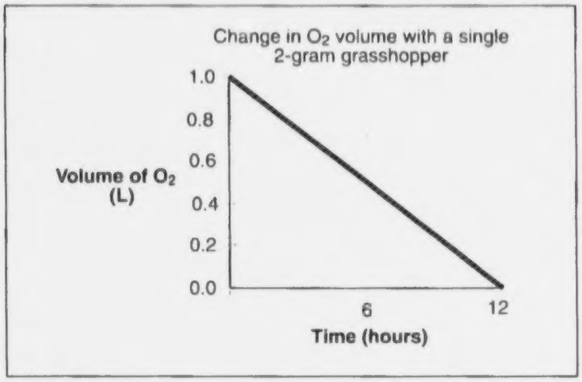
Question

Based on this result, would you predict that 1 grasshopper can survive for 24 hours if sealed inside Biosphere 3? Why or why not?

Answer

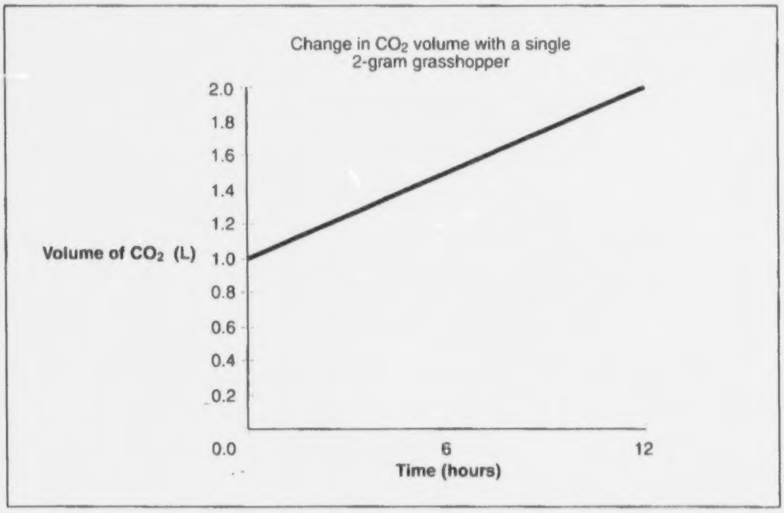
Given the rate at which a 2-gram grasshopper uses O_2, there is only enough O_2 in Biosphere 3 to support the grasshopper for 12 hours. Thus, our model predicts that the grasshopper would not be able to live inside Biosphere 3 for 24 hours.

Following this introduction, ask the students to draw a graph that will show what happens to the amount of O_2 inside Biosphere 3 during the course of the above experiment. Sample results are shown in Graph A.



Graph A. Amount of oxygen in Biosphere 3 containing 1 grasshopper.

Draw a second graph to show what happens to the level of CO_2 in Biosphere 3 during the experiment if 1 L CO_2 were present at the start of the experiment and grasshoppers produce 1 L CO_2 per gram of body mass per day. Sample results are shown in Graph B for Biosphere 3 with a single 2-gram grasshopper.



Graph B. Amount of carbon dioxide in Biosphere 3 containing 1 grasshopper.

HYPOTHESIS GENERATION

Ask students how they would develop a mathematical model of a biosphere that contained grasshoppers, but no plants. The following questions may be used to generate a mathematical equation to describe how the volume of oxygen present changes over time in relation to the number of grasshoppers present in Biosphere 3.

1. What is the initial volume of oxygen in Biosphere 3?
2. What is the relationship between grasshopper body mass and oxygen used?
3. What is the mass of the grasshopper?
4. How much oxygen is used in 1 day by 1 grasshopper?
5. Is there enough oxygen for 1 grasshopper to survive for 1 hour in Biosphere 3? How would you write a mathematical equation to determine this?
6. What is the reasoning behind your prediction?

Sample Hypothesis

If 1 grasshopper is sealed inside Biosphere 3 with 1 L O_2, it will survive for 1 day.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by related test questions and answers for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each group of students the opportunity to expand on the Core Hypothesis.

Question

Can the volume of oxygen in Biosphere 3 be determined if the rate of oxygen consumption by grasshoppers is known?

Hypothesis for Equation Model

If grasshoppers consume 1 L O_2/g/day, then the volume of oxygen in Biosphere 3 at any time "*t*" can be determined mathematically.

Procedure

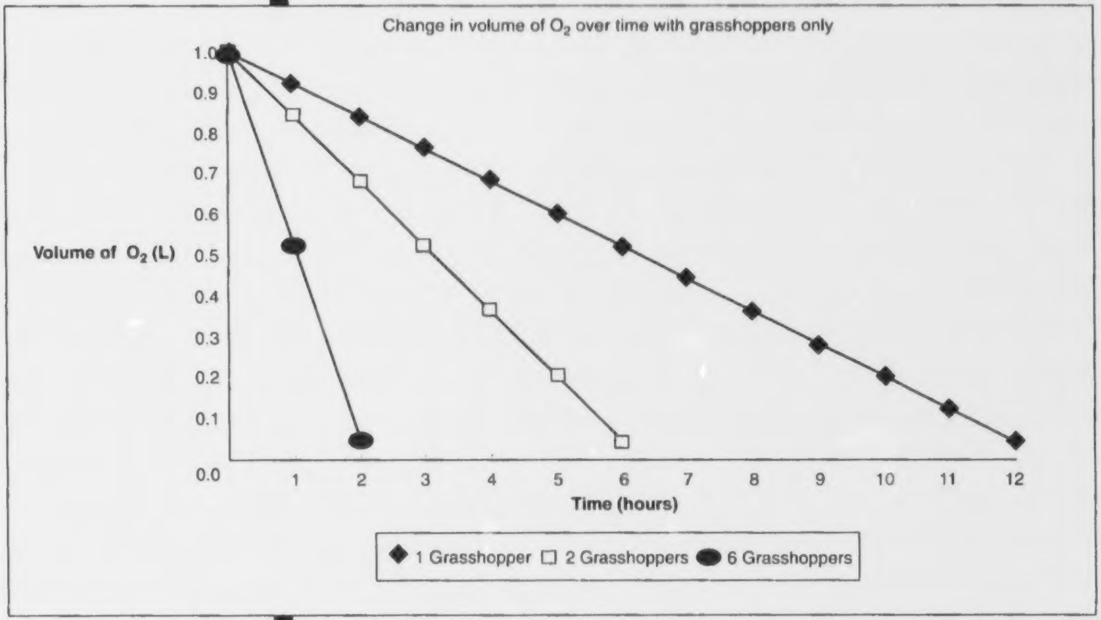
1. Write an equation that describes how oxygen concentration is related to time and to the number of grasshoppers.
2. Roll the die to determine how many grasshoppers your group will seal inside Biosphere 3.
3. Use the equation to determine the change in oxygen volume at 1-hour intervals for the number of grasshoppers sealed in your Biosphere 3.
4. Record your results in a data chart.
5. Write a second equation to show what happens to carbon dioxide concentration.
6. Calculate mathematically what happens to the CO_2 volume within Biosphere 3 within 12 hours, based on the number of grasshoppers sealed in your container. Record your results in a chart.
7. Graph your mathematical conclusions for a 12-hour period.

TEACHER'S NOTES

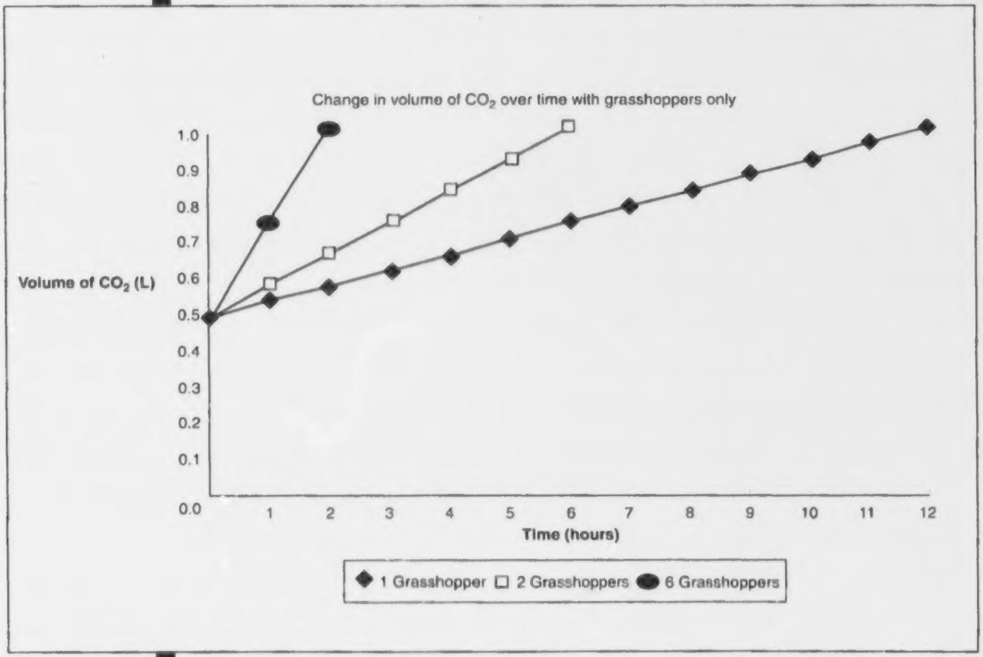
SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Students should graph change in volume of O_2 and CO_2 inside Biosphere 3 over 12 hours. Each group will model a different number of grasshoppers to produce an array of graphs to show class predictions. See Graphs C and D.



Graph C. Change in Biosphere O_2 volume over time with grasshoppers only.



Graph D. Change in Biosphere CO_2 volume over time with grasshoppers only.

TEST QUESTIONS

1. Based on starting assumptions, what changes occurred over the 12-hour period in Biosphere 3?
2. What was the effect on the grasshoppers?
3. What variables have been excluded from the model that might really be important?
4. Why does the volume of carbon dioxide rise and the volume of oxygen fall in Biosphere 3?
5. What happens to the rate of oxygen used as the number of grasshoppers is increased?

STUDENT DESIGN OF THE NEXT EXPERIMENT

Class discussion about the results of the Core Experiment should lead to student questions for developing additional mathematical models for Biosphere 3. Possible questions include:

- What happens if both grasshoppers and plants are in Biosphere 3 at the same time?
- How will respiration rates be affected in Biosphere 3 if the temperature increases?
- What effect will changing periods of light have on photosynthesis?
- Will plants consume the same volume of CO_2 daily as they produce?
- Will the rate of CO_2 production on a per gram body weight basis for a grasshopper be greater than that of a bean plant?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p.15 of the introduction of this book for information on ordering **FREE** copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

This experiment is too detailed to have some exceptional students rely on laboratory partners to explain the setup and communicate observations. The teacher should request the experiment be brailled well in advance of the time it will be used in the classroom. The material in the Teacher Background Content Information should be included.

- Have the student use a computer with graphing capabilities. If one is not available, braille graph paper can be used. If braille graph paper is unavailable, students can make it on their braille writing machine.
- Have students devise their own braille symbols for the long words in the equations.
- Provide die that are large enough to have dots which can be discerned by touch for toss of die.
- Provide print enlargers with a large viewing screen for students who are visually impaired but do not read braille. They could make or purchase large size graph paper or use braille graph paper.
- Construct graphs for the entire class that can be viewed by sight or by touch using a 4-foot square of Pegboard™. Golf tees may be notched with a different shape for each data point. The data points may be connected with string, yarn, ribbon, or cord of various colors and textures.

Answers to Test Questions

1. When 1 grasshopper was sealed inside Biosphere 3, CO_2 volume increased, doubling in 12 hours. O_2 was depleted at the end of 12 hours.
2. At the end of 12 hours, the grasshopper has no O_2 for respiration and it can be predicted that it will die.
3. The needs of the grasshoppers for food and water were ignored. Lack of water, especially, might be important in determining whether the grasshoppers will survive.
4. The volume of carbon dioxide rises and the volume of oxygen falls in Biosphere 3 as a result of cellular respiration by the grasshoppers.
5. As the number of grasshoppers is increased, the rates of carbon dioxide production and oxygen use both increase.

Answers to Questions and Analysis on Student Page

1. A possible conclusion is that models can predict changes in systems.
2. Answers will vary with student models.
3. A computer program that will predict the weather or model global warming will have to consider a multitude of variables and interactions.
4. Changes in temperature would affect respiration rates.
5. Answers will vary with the software used for comparison. Overall, students should come to the conclusion that larger quantities of data and more complex systems may be studied more easily with computer models.

TEACHER'S NOTES

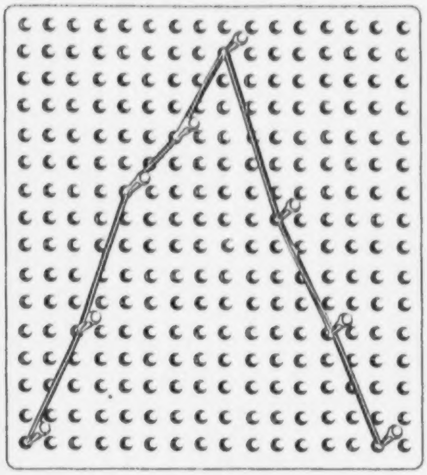


Figure 2. Pegboard™ graph for class use.

Deaf or Hard-of-Hearing

- This experiment presents no new problems. There will be considerable discussion necessary so communication skills are necessary. When there is a large amount of material to be covered in lecture or in question and answer, a signer may be helpful for deaf students if they so desire.

Gifted

- Perform statistical analyses of results.

Mobility Impaired

- Students with manual impairments will need assistance from their instructor and laboratory partners in performing this experiment. Their laboratory group might use the Pegboard™ graph.
- Provide access to tape recorders for students to record the teacher's directions, student discussion, and laboratory partner's comments.

REFERENCES AND SUGGESTED READINGS

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POSSIBLE SOURCES OF MENTORS

- Local college or university statistics or computer modeling professor
- Local research or industry that uses mathematical models

VARIATIONS OF THE CORE EXPERIMENT

TEACHER'S NOTES

After completing the Core Experiment, students should use the results to develop a variation of the experiment. The following directions are intended only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

VARIATION 1

A Plant Kingdom—All Bean Plants, No Grasshoppers

SYNOPSIS

Students will develop a mathematical model of the relationship between plants and oxygen supply in a closed system.

HYPOTHESIS GENERATION

Question

What is the relationship between oxygen supply in a closed system and the survival of plants?

Sample Hypothesis for Equation Model

If plants consume 0.5 L O_2/g/day and produce 0.7 L O_2/g/day, then the volume of O_2 in Biosphere 3 at any time "*t*" can be determined mathematically.

Sample Experimental Procedure

1. Write an equation that describes how oxygen volume is related to time and to numbers of plants. A possible equation is:

Oxygen volume in Biosphere 3 = Starting volume - Oxygen used + Oxygen produced where

$$O_2 \text{ used} = \frac{0.5\text{ L}}{\text{gram/day}} \bullet \frac{\#\text{ grams}}{\text{plant}} \bullet \#\text{ plants} \bullet \#\text{ hours} \bullet \frac{1\text{ day}}{24\text{ hours}}$$

and

$$O_2 \text{ produced} = \frac{0.7\text{ L}}{\text{gram/day}} \bullet \frac{\#\text{ grams}}{\text{plant}} \bullet \#\text{ plants} \bullet \#\text{ hours} \bullet \frac{1\text{ day}}{24\text{ hours}}$$

Since more O_2 is produced than is used by each plant, combining the O_2 used and the O_2 produced gives

$$O_2 \text{ volume} = 1\text{ L} + \frac{0.2\text{ L}}{\text{gram/day}} \bullet \frac{\#\text{ grams}}{\text{plant}} \bullet \#\text{ plants} \bullet \#\text{ hours} \bullet \frac{1\text{ day}}{24\text{ hours}}$$

which represents an increasing volume of O_2 over time.

TEACHER'S NOTES

If all plants are 10 grams, this model reduces to

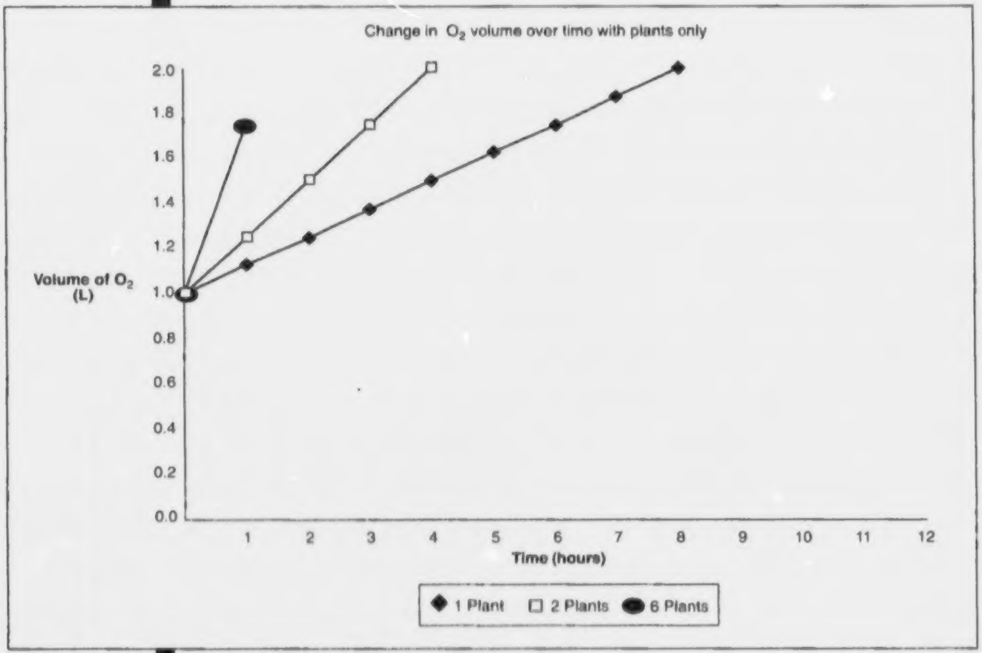
$$O_2 \text{ volume} = 1 \text{ L} + 0.2 \text{ L} \bullet 10 \bullet \# \text{ plants} \bullet \frac{\# \text{ hours}}{24 \text{ hours}}$$

2. Roll the die to determine how many bean plants your group will seal inside Biosphere 3.
3. Use the equation to determine the change in O_2 volume at 1 hour intervals for the number of plants sealed in Biosphere 3.
4. Record your results in a chart.
5. Write a second equation to show what happens to the carbon dioxide volume.
6. Calculate mathematically what occurs to the volumes in your container during a 12-hour period based on the number of plants in your Biosphere 3.
7. Graph your mathematical conclusions for a 12-hour time period.

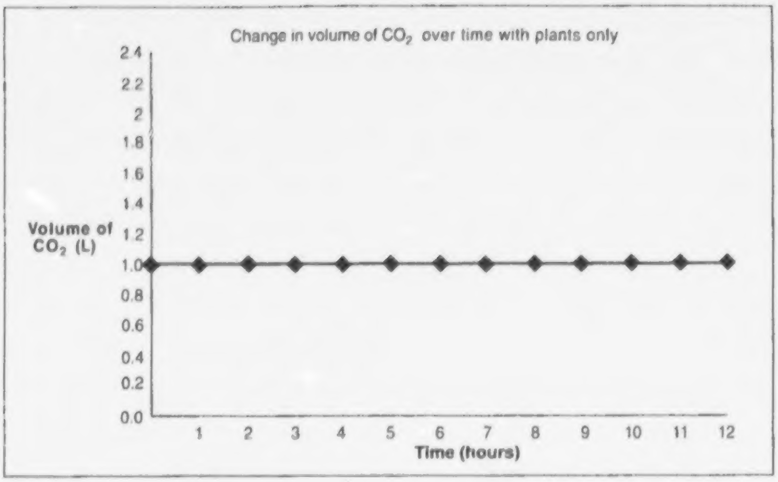
DATA ANALYSIS AND INTERPRETATION

Sample Data

Students should graph change in the volumes of O_2 and CO_2 inside Biosphere 3 over 12 hours. Each group will model a different number of bean plants to produce a range of class predictions. See Graphs E and F.



Graph E. Change in O_2 volume over time with plants only.



Graph F. Change in CO_2 volume over time with plants only.

TEST QUESTIONS

1. Based on starting assumptions, what changes occur inside Biosphere 3 over 12 hours?
2. What was the effect on the bean plants?
3. Is carbon a limiting factor?
4. Why is it necessary to make so many assumptions? Why is it necessary to make Biosphere 3 as simple as it is?

VARIATION 2

An Animal and Plant Kingdom

SYNOPSIS

Both grasshoppers and bean plants are sealed in Biosphere 3 with the assumption that the grasshoppers do not consume the bean plants.

HYPOTHESIS GENERATION

Question

What will be the oxygen consumption when both beans and grasshoppers are sealed in Biosphere 3?

Sample Hypothesis Equation Model

If grasshoppers and bean plants are sealed inside Biosphere 3, then the equations for their oxygen consumption can be combined for a mathematical model of their combined effect. A suggested model is as follows:

O_2 Volume = Starting volume + Net oxygen from grasshoppers + Net oxygen from plants

$$+ 1\text{ L} + \left(\frac{-1\text{ L}}{\text{gram/day}} \bullet \frac{\#\text{ grams}}{\text{grasshopper}} \bullet \#\text{ grasshoppers} \bullet \#\text{ hours} \bullet \frac{1\text{ day}}{24\text{ hours}}\right)$$

$$+ \left(\frac{0.2\text{ L}}{\text{gram/day}} \bullet \frac{\#\text{ grams}}{\text{plant}} \bullet \#\text{ plants} \bullet \#\text{ hours} \bullet \frac{1\text{ day}}{24\text{ hours}}\right)$$

Answers to Test Questions

1. CO_2 volume is constant, but O_2 volume increases by a factor of 0.125 L/hr.
2. Bean plants will continue to grow.
3. This should not be a factor in this model as CO_2 volume remains the same.
4. Discussion of this question should lead students to realize that even the simple Biosphere 3 model becomes complicated very quickly as variables are added.

TEACHING TIP

This model is a combination of the two models developed previously. Ask students how the two previous equations for oxygen change with grasshoppers and plants could be used to describe the change in oxygen volumes inside Biosphere 3 at any time "t" if both grasshoppers and bean plants are sealed inside.

TEACHER'S NOTES

and for grasshoppers of 2 grams each and bean plants of 10 grams each this model becomes

$$O_2\ \text{Volume} = 1\text{ L} - \left(1\text{ L} \bullet 2 \bullet \#\text{ grasshoppers} \bullet \frac{\#\text{ hours}}{24\text{ hours}}\right) + \left(0.2\text{ L} \bullet 10 \bullet \#\text{ plants} \bullet \frac{\#\text{ hours}}{24\text{ hours}}\right)$$

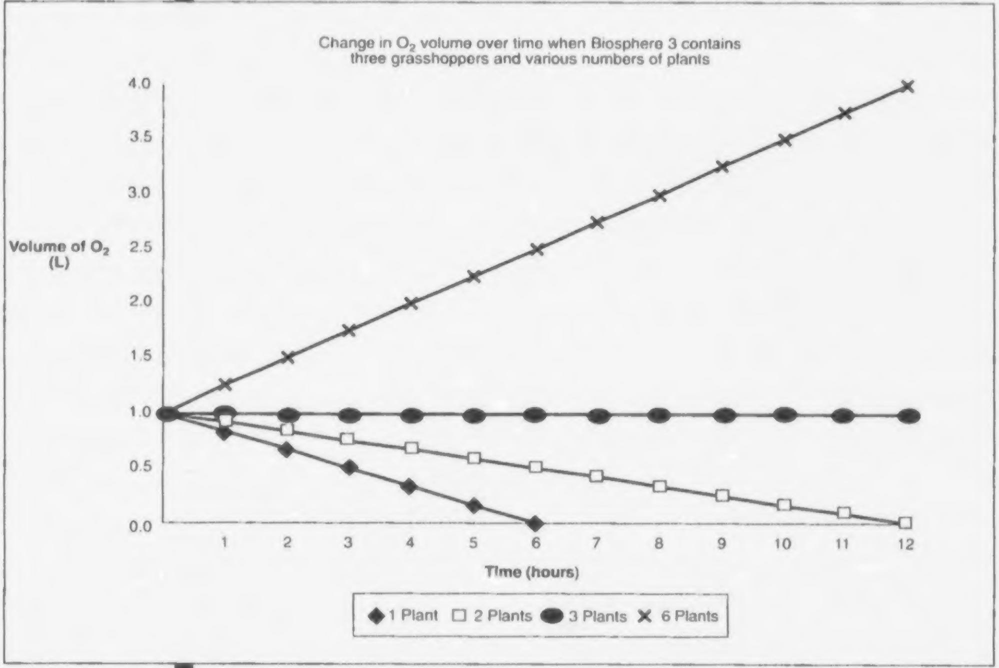
Sample Experimental Procedure

1. Write a single equation to describe the effect of both grasshoppers and bean plants on oxygen volumes inside Biosphere 3.
2. Roll the die to determine how many grasshoppers your group will seal inside Biosphere 3.
3. Roll the die again to determine how many bean plants your group will seal inside Biosphere 3.
4. Calculate mathematically what occurs with the O_2 and CO_2 volumes for a 12-hour period.
5. Graph your calculations.

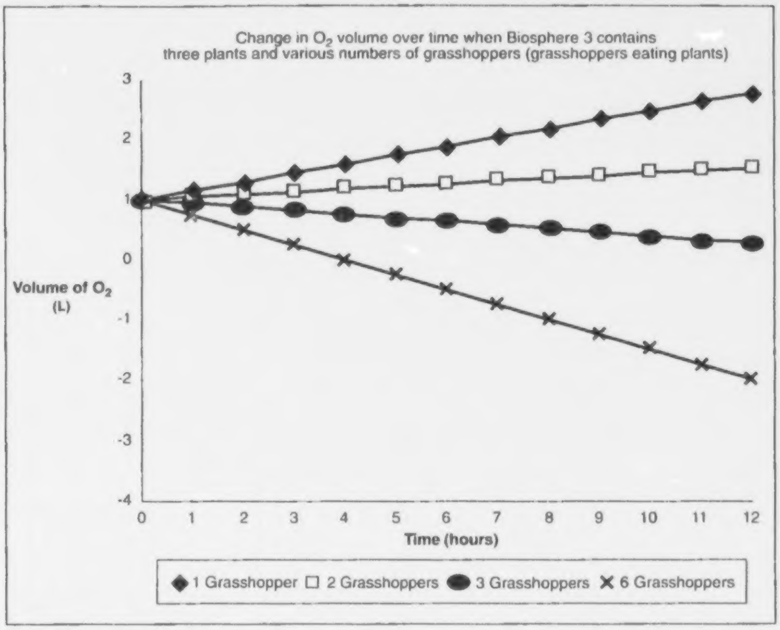
SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Students might graph change in volume of O_2 inside Biosphere 3 over a 12-hour period as shown below.



Graph G. Change in O_2 volume over time with 3 grasshoppers and variable numbers of plants.



Graph H. Change in O_2 volume over time with 3 plants and variable numbers of grasshoppers.

TEST QUESTIONS

1. Based on starting assumptions, what change or changes occurred over 12 hours in Biosphere 3?
2. What effect was there on the grasshoppers?
3. What effect was there on the bean plants?
4. When there are 6 grasshoppers, does the model reflect what will really happen over 12 hours?

VARIATION 3

Another Look at the Animal and Plant Kingdom

SYNOPSIS

Both grasshoppers and bean plants are sealed in Biosphere 3, with the assumption that the grasshoppers do eat the bean plants.

ADDITIONAL INSTRUCTIONAL PROCEDURES

This variation requires the following additional assumptions:

- Each grasshopper grows at a rate of +0.1 g/day.
- Bean plants grow at a rate of +0.1 g/day.
- Grasshoppers eat 2 g plant tissue/day/grasshopper.

HYPOTHESIS GENERATION

Question

What will be the oxygen consumption when both beans and grasshoppers are sealed in Biosphere 3 and grasshoppers can eat plants?

Sample Hypothesis Equation Model

If grasshoppers and bean plants are sealed inside Biosphere 3 with the grasshoppers

Answers to Test Questions

1. Only when bean plants equal or outnumber grasshoppers does O_2 remain constant or rise in Biosphere 3.
2. Whenever grasshoppers outnumber plants, oxygen is depleted in Biosphere 3.
3. Since plants require oxygen too, low levels will harm them also.
4. No, since after 4 hours, all the oxygen will be gone. The graph beyond 4 hours is incorrect. All plants and grasshoppers will die when all oxygen is depleted. Oxygen levels cannot be negative in reality even if our mathematical model says they can be.

TEACHING TIP

Approach to modeling—The same strategy used earlier to develop a model is applied again. Each term in the model that follows describes one of the effects on oxygen volume, such as the change in oxygen volume inside Biosphere 3 at any time "*t*" where P represents plants and G represents grasshoppers.

eating the plants, then the equations for their oxygen consumption can be combined for a mathematical model of their combined effect. A suggested model where P = # plants and G = # grasshoppers is as follows:

$$1\ \text{L}\ O_2 + [P \bullet 10\ \text{g/plant} \bullet (0.7\ \text{L}\ O_2 - 0.5\ \text{L}\ O_2)/\text{g} \bullet t\ \text{hours/24 hours}] - [G \bullet 2\text{g/grasshopper} \bullet 1\ \text{L}\ O_2/\text{g} \bullet t\ \text{hours/24 hours}] - [G \bullet 2\ \text{g/grasshopper} \bullet (0.7\ \text{L}\ O_2 - 0.5\ \text{L}\ O_2)/\text{g} \bullet t\ \text{hours/24 hours}] - [G \bullet 0.1\ \text{g} \bullet 1\ \text{L}\ O_2/\text{g} \bullet t\ \text{hours/24 hours}] + [P \bullet 0.1\ \text{g} \bullet (0.7\ \text{L}\ O_2 - 0.5\ \text{L}\ O_2)/\text{g} \bullet t\ \text{hours/24 hours}]$$

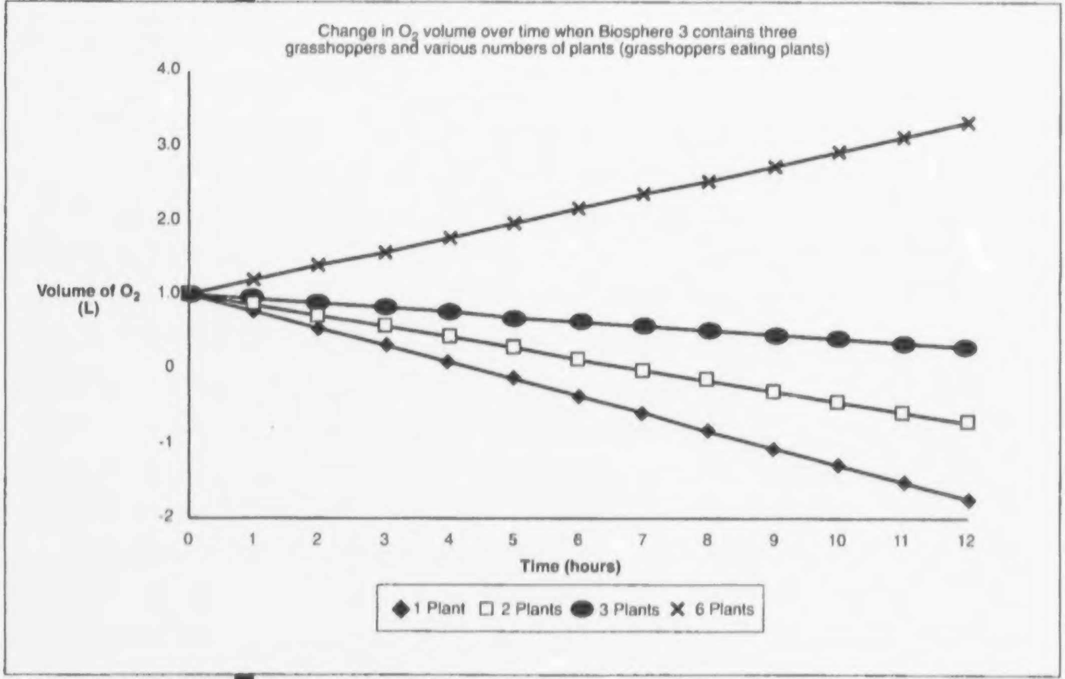
Sample Experimental Procedure

1. Write an equation that describes the effect on oxygen volume of all of the following: a) the presence of plants, b) the presence of grasshoppers, c) animals eating plants, d) animals growing—thus consuming more oxygen, e) plants growing—thus producing more oxygen.
2. Roll the die to determine how many grasshoppers your group will seal inside Biosphere 3.
3. Roll the die again to determine how many bean plants your group will seal inside Biosphere 3.
4. Calculate mathematically what occurs with the O_2 and CO_2 concentrations for a 12-hour period.
5. Graph your calculations.

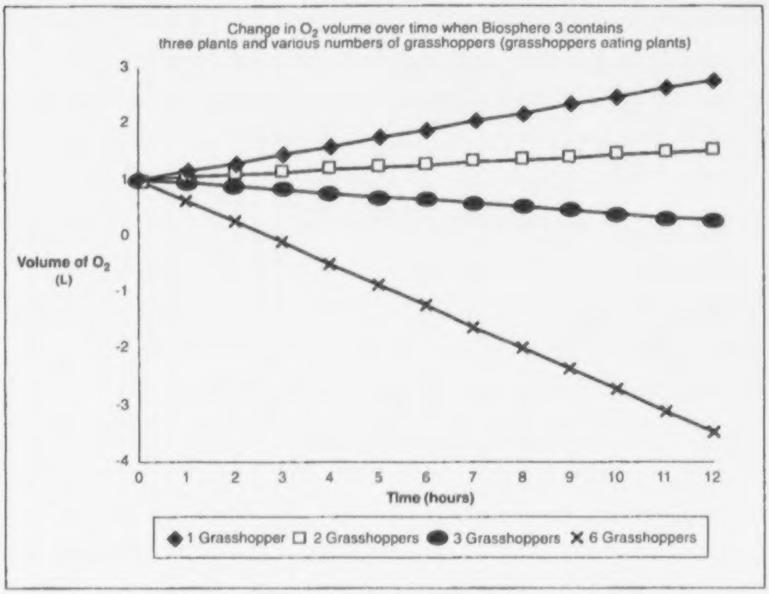
SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Students should graph change in the volume of O_2 inside Biosphere 3 over a 12-hour period (see Graphs I and J).



Graph I. Change in O_2 volume over time with 3 grasshoppers eating plants and a variable number of plants.



Graph J. Change in O_2 volume over time with 3 plants and a variable number of grasshoppers eating plants.

TEST QUESTION

Based on starting assumptions, what will change inside over time?

Answer to Test Question

Oxygen will be depleted unless the plants outnumber the grasshoppers. When all oxygen is depleted, all plants and grasshoppers will die. Thus, the Graphs I and J do not reflect reality when the volume of oxygen goes below 0.

Biosphere 3 — It's a Small World After All

Directions for Students

INTRODUCTION

Our biosphere consists of many systems that interact with each other. Among the factors that determine what kinds of organisms can survive in a particular place are temperature, rainfall, and light.

All life forms are connected with each other and interact with the non-living or abiotic environment. Photosynthetic bacteria and plants contribute oxygen to the atmosphere and remove carbon dioxide; transforming energy from the sun, they lock it into organic compounds. Animals gain energy by feeding on plants and each other. Together, biotic and abiotic components make up an ecosystem, where nutrients, water, oxygen, carbon, and nitrogen are constantly being recycled.

Change occurs in ecosystems when organisms modify their environment, both over the short and long term. In this experiment, you will develop a mathematical model of an ecosystem and predict CO_2 and O_2 concentrations and the effects of their change on plant or animal populations. Models are useful tools for biology in general and for ecology in particular. Systems as complicated as biospheres are difficult to study without the help of simplified models or simulations.

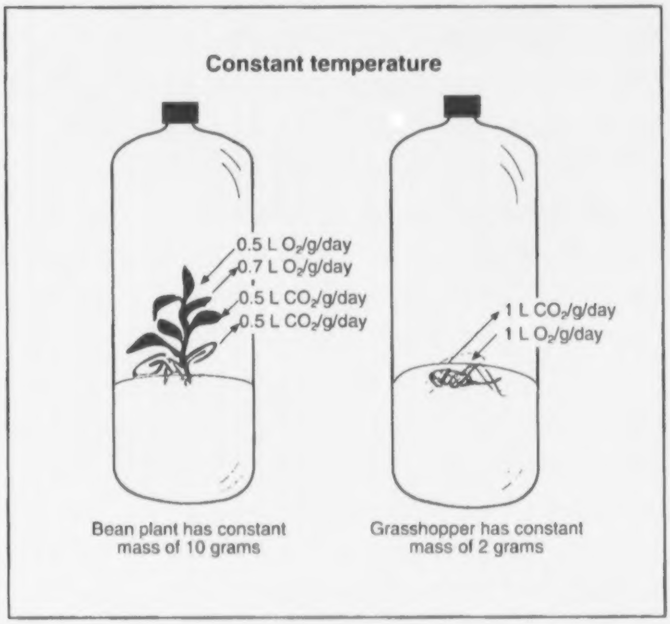


Figure 1. Biosphere 3.

OBJECTIVES

At the end of this lab, you should be able to:

- Develop a simple mathematical model that investigates the complicated processes and systems of an artificial ecosystem.
- Describe the role each biotic and abiotic variable plays within the ecosystem.
- Observe, record, and interpret how different components of the ecosystem change as they interact with each other and the environment.
- Discuss how models are used to investigate complicated processes and systems.
- Discuss the difficulties of controlling all variables in a complicated system.

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of potential nutritional mutants. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 4 sheets of graph paper
- 1 calculator
- 1 number cube or die
- 1 or more class "closed bottle" systems (*optional*)

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested by a mathematical model which generates quantitative data. Explain the reasoning behind your hypothesis.

Answer the following questions:

1. What is the question you are investigating?
2. How are variables controlled in your experiment?
3. What assumptions are built into your model?
4. How do you know whether your assumptions are good ones?

PLAN OF INVESTIGATION

Below, write the equation for the mathematical model you will use in your experiment. Briefly describe what it means. Use your model to generate data that will test your hypothesis. Analyze your data and discuss the results with the class.

You must have your teacher approve this protocol before you begin the experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. What conclusions can you reach about your hypothesis?
2. How do the predictions made by your model apply to the real world?
3. Judging from how complicated the Biosphere 3 model becomes when just a few variables are included, comment on the difficulties of writing a computer program that will predict the weather or model global warming.
4. Predict the effect of temperature change in Biosphere 3 on respiration rates.
5. Compare software such as *BioBlast*™ to mathematical models developed in this activity.

DESIGN OF VARIATIONS OF CORE EXPERIMENT

After collecting and analyzing the data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for models that you could do next. Think of questions that occurred to you as you conducted the Core Experiment. Questions other students have studied include the following:

- What is the relationship between oxygen supply in a closed system and the survival of plants?
- What will be the oxygen consumption when both beans and grasshoppers are sealed in Biosphere 3?
- What will be the oxygen consumption when both beans and grasshoppers are sealed in Biosphere 3 and grasshoppers can eat plants?

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NOTES

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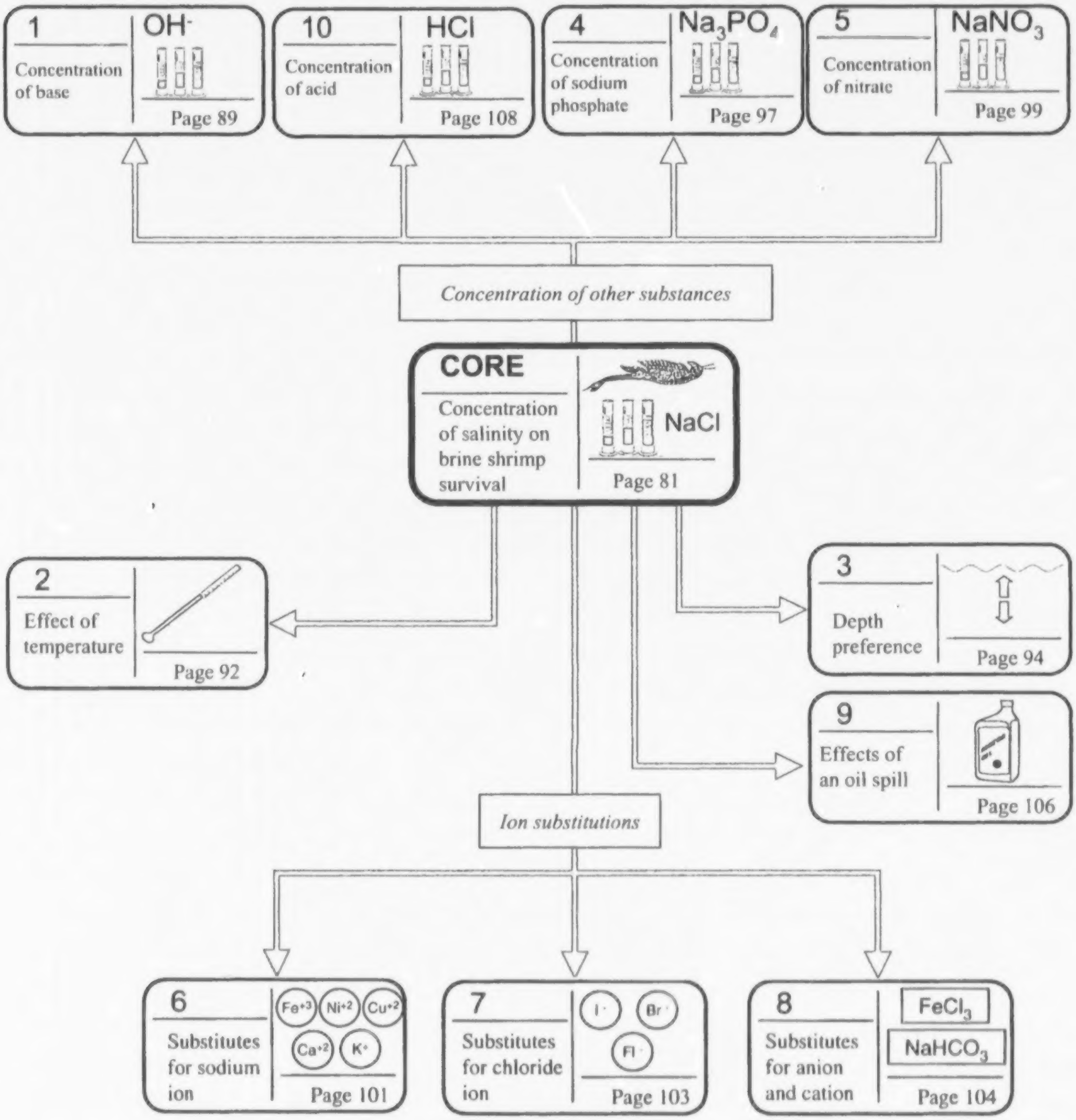
Tolerance Testing With a Hairless "Guinea Pig"

At-A-Glance Map of the Core Experiment and Its Variations


1
OH-
Concentration of base
Page 89
10
HCl
Concentration of acid
Page 108
4
Na3PO4
Concentration of sodium phosphate
Page 97
5
NaNO3
Concentration of nitrate
Page 99
Concentration of other substances
CORE
Concentration of salinity on brine shrimp survival
NaCl
Page 81
2
Effect of temperature
Page 92
3
Depth preference
Page 94
9
Effects of an oil spill
Page 106
Ion substitutions
6
Substitutes for sodium ion
Fe+3
Ni+2
Cu+2
Ca+2
K+
Page 101
7
Substitutes for chloride ion
I-
Br-
Fl-
Page 103
8
Substitutes for anion and cation
FeCl3
NaHCO3
Page 104

NOTES

Tolerance Testing With a Hairless "Guinea Pig"

SYNOPSIS FOR CORE EXPERIMENT

Students will explore the tolerance of brine shrimp (*Artemia franciscana*, formerly *Artemia salina*) to various concentrations of NaCl. They will determine the effect of increased salinity on the survival of *A. franciscana*.

APPROPRIATE BIOLOGY LEVEL

Introductory, advanced, marine science

TEACHER PARTNERS

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Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 89. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this research lab, students will be able to:

- Define and determine the tolerance limit of *Artemia franciscana* larvae to environmental variables, such as salinity.
- Describe how an environmental variable may be a limiting factor in an ecosystem and act as a selective pressure in the survival of a species.
- Develop the skill of using a serial dilution to prepare various concentrations of solutions.

MATERIALS NEEDED

For the teacher preparation, you will need the following for a class of 24:

- 500 adult brine shrimp or 3 teaspoons of brine shrimp (*Artemia franciscana*) eggs
- 5 clean, plastic 3.8-L (1-gallon) milk jugs
- 200 g NaCl, non-iodized table, canning, or pickling salt
- 20 L distilled water
- 9 culture/dilution containers

For the teacher-led preliminary introduction, you will need the following for a class of 24:

- 500 adult brine shrimp or 2-day-old culture of brine shrimp with 200 eggs and larvae
- 1 1-L beaker
- 500 mL 1.0% NaCl solution
- 1 clean, white margarine container
- 1 overhead projector or dissecting microscope with video camera
- 5 mL ammonia
- 5 mL liquid soap
- 5 mL spray cleaner
- 5 mL vegetable oil
- 5 mL vinegar
- 5 mL liquid fertilizer
- 5 g road de-icer
- 1 tissue culture plate (*optional*)

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Introduce the lab with a discussion of the law of tolerance, the concept of limiting factors in the ecosystem, and the natural history of *Artemia*. Brainstorm and research ideas for hypotheses.

Day 2 (30 minutes)

- Explain serial dilutions. (15 minutes)
- Have students practice serial dilutions.

Day 3 (45 minutes)

- Observe brine shrimp.
- Develop hypotheses and experimental design.
- Set up the lab.

Day 4 (30 minutes)

- Collect data and analyze.

Day 5 (45 minutes)

- Interpret these data. Generate hypotheses for variations and develop experimental design.

PREPARATION TIME REQUIRED

30 to 45 minutes

- Purchase live brine shrimp or set up a hatchery for brine shrimp. Prepare stock solutions.

15 to 20 minutes

- Gather additional supplies for introduction and student experiments.

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TEACHER'S NOTES

You will need the following for each group of two to four students in a class of 24:

- 1 10-mL disposable syringe or 10-mL graduated pipette with bulb
- 1 permanent marker
- 10 translucent film cans, 50-mL beakers, test tubes, or 1 divided box with 10 chambers
- 1 teasing needle
- 1 1-mL graduated plastic pipette
- 1 stereo microscope
- 10 mL 8.0% NaCl
- 40 mL distilled H_2O
- 1 dropping pipette
- 1 petri dish with counting grid

SAFETY PROCEDURES

Always wear goggles and lab aprons when working with chemicals.

Label all containers so solutions do not become confused.

Always **add acid to water** when preparing acid solutions.

Use caution when mixing the suggested pollutants during the introduction of the Core Experiment if other "pollutants" are used in addition to those suggested. Be aware of any potential interaction of the chemicals.

Never mix bleach and ammonia or substances containing either substance; toxic fumes will result.

Use only substances that can be poured safely down the sink.

DIRECTIONS FOR SETTING UP THE LAB

Stock Solutions

1.0% NaCl Solution

Dissolve 10 g of salt in 1 L of distilled water. Since a large volume of this brine solution will be needed, you may find it convenient to mix a gallon at a time. Pickling salt is a good choice because it lacks the sodium silico-aluminate added to keep table salt free-flowing. Prepare a gallon by adding 38.8 g NaCl to a well-cleaned milk jug. Fill with distilled water. This is approximately one tablespoon of salt per quart of water (0.25 cup salt per gallon of water); that precision is adequate for the culture stock and as a diluent for the acid.

8.0% NaCl Solution

1. Dissolve 8.0 g NaCl in a small amount of distilled water.
2. Bring to a volume of 100 mL with distilled water.

Brine Shrimp Hatchery (Optional)

1. Tape a piece of acetate parallel to the sides of a sandwich box about 3 cm from one side. Leave a 1-cm gap between the acetate and the bottom of the box. Set the box where it will receive light and be undisturbed. Do not try to move the box after you have added eggs.
2. Fill the box with the 1.0% NaCl stock solution to a depth of at least 3 cm.
3. Sprinkle approximately 3 teaspoons of brine shrimp eggs over the large section of the box. The eggs should be a single layer thick on the surface.

4. Cover the large section, not touching the water, with the opaque lid. The brine shrimp will begin hatching in 1 to 2 days and will swim under the acetate barrier into the small lighted section of the box.
5. Set up additional boxes to produce more larvae. The hatching chamber is shown in Figure 1.

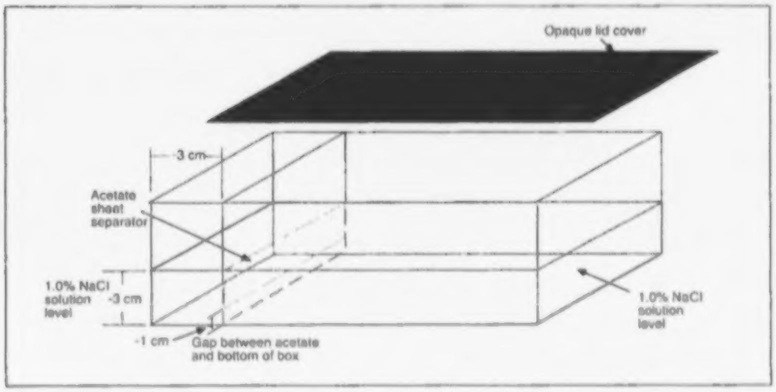

Figure 1. Brine shrimp hatchery.

TEACHER BACKGROUND

Content Information

Brine shrimp (*Artemia*) are members of the class Crustacea and the subclass Branchiopoda (Barnes, 1986). Brine shrimp are not really shrimp, but belong to a separate order (Anostraca) of crustaceans. *Artemia* are known commonly as sea monkeys or fairy shrimp. See Figure 2. While they do require a saltwater environment, they do not live naturally in the ocean. They are found in salt pans and lakes, such as the Great Salt Lake. Fish are rare in these environments, but brine shrimp are able to tolerate these high salt concentration environments. In some western saline bodies of water, brine shrimp occur seasonally in such high numbers they turn the water red or brownish-red. This coloration results from the green algae they eat that appear to turn red in the process of digestion (Schmitt, 1965).

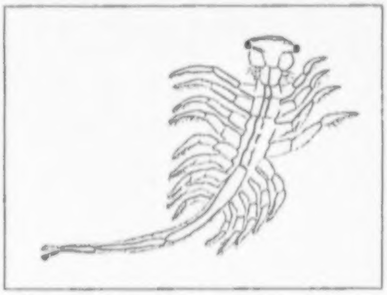

Figure 2. Brine shrimp.

Although brine shrimp can tolerate high salt concentrations, they are very sensitive to changes in their surroundings. Due to this sensitivity, they are useful as an indicator species of the presence of chemicals and temperature fluctuations. Ecologists use indicator species as signals of problematic changes in the ecosystem. Brine shrimp are obtained readily and inexpensively, and the "eggs" hatch quickly. What look like "eggs" are not eggs at all, but cysts containing a dormant gastrula. As unfed larvae, brine shrimp are sensitive to various materials so they can be used as bioindicators of

TEACHER'S NOTES

contamination. They even are used in preliminary screens to detect potential tumor suppressing natural products (Meyer et al., 1982).

Pollution is not always obvious. Water that looks clean and clear may be highly contaminated. Humans are not the only creators of pollution on Earth. Volcanoes produce sulfur dioxide; decaying matter and methanogenic bacteria release methane; naturally occurring forest fires emit carbon dioxide; and lightning creates ozone. Even rain could be considered a pollutant to a saline body of water.

Recycling is not a new concept. All matter is recycled. Although the recycle time for some materials, like phosphates, is very long. An example of this recycling process is the nitrogen cycle. Nitrogen is needed by living things for the formation of amino acids and nitrogenous bases (pyrimidines and purines). Nitrogen is the most abundant gas (about 78%) in the air, but most organisms cannot use gaseous nitrogen directly. Nitrogen-fixing bacteria convert about 139 million tonnes (1 tonne = 1 metric ton) of nitrogen each year and aquatic organisms up to 120 million tonnes (Goldsmith & Hildyard, 1988). Nitrogen is converted into the soluble form, nitrate, that can be absorbed and used by plants. The nitrogen in plants can be used by animals that consume them. When the animal dies, bacteria and fungi further use the nitrogen and release it in the form of ammonia. The ammonia may be further used by other kinds of bacteria, ultimately being oxidized to gaseous nitrogen and released into the atmosphere. Human activities have altered the fluxes in this cycle. The application of artificial fertilizers releases at least 55 million tonnes of nitrogen per year. Power plants and automobiles contribute an additional 24 million tonnes in the form of nitrous oxides. Excessive amounts of these forms of nitrogen are partially responsible for environmental degradation by acid rain. Brine shrimp can be used as indicators of these forms of pollution as shown in the Variations of the Core Experiment.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts:

Correct Concept	Misconception
• Water that looks clean and safe to drink may contain harmful pollutants.	• Water that looks clean is safe to drink.
• Water pollution is threatening the health of the Earth's waterways and oceans.	• Water pollution is not really a problem because the water and insensitive aquatic organisms can recycle these substances.
• Some substances, even in small quantities, can be hazardous to some organisms, while other substances are only toxic in large quantities and/or to certain organisms.	• If a substance is toxic, even minute amounts of it are toxic.
• Some organisms are adapted to extreme conditions and/or a wide range of environments while others are very sensitive to environmental change.	• What is unhealthy for one organism is unhealthy for all organisms.
• Different organisms have different ranges of tolerance for various chemical and physical factors.	
• Tolerance to one abiotic factor or pollutant does not presuppose tolerance to all factors	• "Good" conditions for one organism will be "good" for all organisms.

TEACHING TIPS

- Purchased brine shrimp are larger and easier to examine. It also reduces the preparation time to prepare and maintain a culture.
- Distinguish between naturally fluctuating abiotic factors and introduced pollutants.
- Always have a supply of brine shrimp that are freshly hatched. If not purchasing brine shrimp, start one culture on Friday before leaving school and another one on Monday afternoon.
- Always keep the brine shrimp culture aerated with a gentle bubble stream or in containers with a large surface area.
- Feed the brine shrimp larvae with baker's yeast and the adults with dry, powdered fish food. *Dunaliella*, a unicellular alga, and whole wheat flour also can be used to rear larvae to the adult stage.
- Brine shrimp can be concentrated by covering the end of a pipette with fragrance-free tissue or fine, clean fabric and suctioning off excess water.
- Students may want to recognize brine shrimp health in more categories than live and dead. They will see some brine shrimp that are alive, but unable to swim in a direct line, merely twitching or swimming in small circles. For the Core Experiment and all variations except Variation 4, the disabled shrimp have been counted with the dead. Variation 4 data illustrate results students might obtain if they recognize the three categories of health.
- Expect responses to a range of concentrations of potential pollutants not to be linear.
- Emphasize the assay potential of this exercise rather than accuracy in creating dilutions.
- The results are quite similar if 8 mL of stock potential pollutant are used with 2 mL of diluent and 1 mL of brine shrimp, or if 8 mL of stock potential pollutant are used with 1 mL of diluent and 1 mL of brine shrimp.
- Keep the dilution math simple. Syringes make the process of making the dilutions very easy. You then can emphasize the concept rather than the manipulation. If

(continued on p. 81)

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Table 1. Directions for salt concentration preparations.

Variation	Inorganic compound name	Molecular formula	Molecular weight (g/mole)	Amount (g) to make 100 mL of 0.17N (equivalent) solution	Normality (equivalence) of 1.0% solution
1	Sodium hydroxide	$NaOH$	40.01	0.6802	0.25
4	Sodium orthophosphate	$Na_3PO_4 \bullet 12H_2O$	380.16	6.4627	0.17
5	Sodium nitrate	$Na(NO_3)_2 \bullet 6H_2O$	184.37	3.1343	0.05
6	Potassium chloride	KCl	74.55	1.2674	0.13
	Calcium chloride	$CaCl_2$	110.99	1.8868	0.09
	Nickel chloride	$NiCl_2$	129.60	2.2032	0.08
	Lead chloride	$PbCl_2$	278.12	4.7280	0.04
	Ferrous (iron) chloride	$FeCl_2$	126.76	2.1549	0.08
7	Sodium bromide	$NaBr$	102.91	1.7495	0.10
	Sodium iodide	NaI	149.92	2.5486	0.07
	Sodium fluoride	NaF	42.00	0.7140	0.24
8	Sodium hydrogen carbonate	$NaHCO_3$	84.02	1.4283	0.12

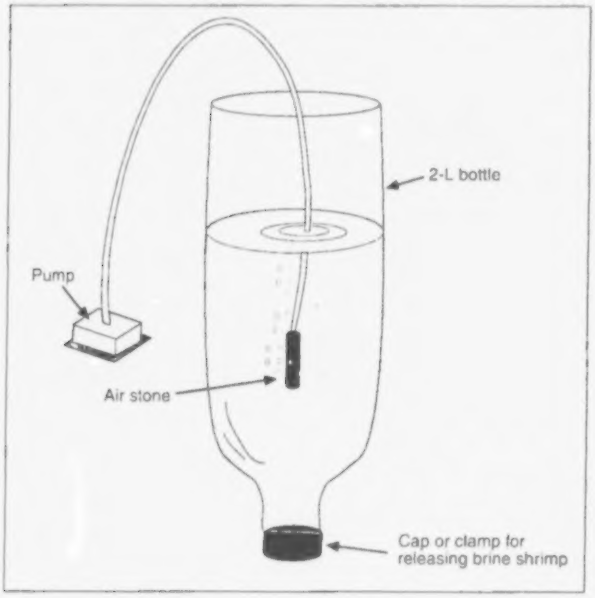


Figure 3. Simple brine shrimp hatchery using a 2-L soda pop bottle.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Introduce the class to brine shrimp by placing a small amount of the stock brine shrimp culture into a petri dish on an overhead projector or a dissecting microscope with video camera. Have the class observe the eggs, larvae, and adult brine shrimp. Information about the life history of brine shrimp is important because it will lead students to consider that the impact of the test materials may vary with the maturation of the organism. Discuss the economic importance of brine shrimp and their increasing use in fast, simple, and inexpensive tests to study pollution and potential cytotoxicity.

It is often very difficult to study the effects of a particular abiotic factor in nature. Variables in nature are not controlled easily. Many factors occur simultaneously and some may interact with each other. Once a problem is detected, the specific cause

TEACHING TIPS

(continued from p. 80)

you make a dilution series from 1-in-100 to 1-in-1000 to 1-in-10,000 or a series from 1-in-100 (1.0%) to 1-in-200 (0.5%) to 1-in-400 (0.25%), the preparation and the concept will be emphasized in repetition. Recall that pollutants usually are reported in parts per million (ppm) or parts per thousand (ppt), and 1 ppm is 0.0001%.

- Practice making serial dilutions with a dye, such as methylene blue. Use the spectrophotometer to provide quantitative data for solution concentrations.
- Consider the advantage of using Multiwell™ or similar tissue culture plates. They are stable and not easily tipped or spilled. The different dilutions are stored and retrieved easily and some are prelabeled. Brine shrimp can be observed easily by setting the plate on the microscope stage. Each well in a plate with 24 wells holds just 2.5 mL of test solution plus 0.25 mL of dense brine shrimp culture. Each well in a six-well plate holds 15 mL.
- You may want to reduce the range of dilutions used in a variation. An efficient way to do this is to zero in on the critical range where some, but not all of the organisms, are affected. Test extremes with single samples. Replicate the trials where a difference in mortality is detected.
- Although the hypothesis may be phrased in terms of mortality or survival, the more common way to phrase the results is mortality if you are testing the detrimental effects of a potential pollutant.
- Table 1 contains preparation directions for creating salt concentrations of the same equivalence (normality) (.17N) as 1.0% sodium chloride. See Table 1.

(continued on p. 82)

may not be evident. Conducting a laboratory experiment under controlled conditions is one way to isolate the effects of a single variable on a specific organism. The results can be used to make inferences about the impact of this variable in nature.

Discuss the harsh environment where brine shrimp are found and how they deal with the abiotic factors. Does a wide range of tolerance for one factor suggest a similar tolerance for all factors?

Fill a 1-L beaker half full with the saline solution. Tell the students this represents a small ecosystem where brine shrimp might live. If you have a video camera that fits your dissecting microscope, you might do this entire demonstration in the wells of a tissue culture plate. Place a small amount of pollutant, such as ammonia, liquid soap, vegetable oil, vinegar, liquid fertilizer, road de-icer, or spray cleaner, into the jar. Discuss how each of the pollutants may end up in the environment and how they may affect the organisms living there.

Ask students to consider the source and nature of pollutants.

- Who or what is responsible for releasing pollutants into the environment?
- Are any naturally occurring materials pollutants?
- What impact will the addition of these materials have on the living organisms in the environment?
- How much of each material will it take to harm the environment?
- How long will each material stay in the environment?

Ask the students to speculate about the survival of brine shrimp if they are exposed to variations of a key abiotic factor. Pose the following questions:

- Would variations of a key abiotic factor cause the brine shrimp to die?
- Could a small variation of a key abiotic factor be harmless to the brine shrimp?
- How could you design an experiment to test the effects of specific variations in key abiotic factors on living organisms?

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

The hypothesis for the Core Experiment is based on tolerance levels to changes in the environment. The students should consider what would happen if there were an increase or decrease in the salinity of the brine shrimp's environment. There are two possibilities. The addition of NaCl could cause a change in the survival rate or there could be no measurable effect on their survival. An increase in the survival rate would not be noticeable during a short observation period.

Sample Hypotheses

- An increase in the NaCl concentration will cause a decrease in the reproductive rate of the brine shrimp.
- The addition of a small amount of NaCl will kill all the brine shrimp.
- The adult brine shrimp will be able to survive a higher concentration of NaCl than the larvae.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that

TEACHING TIPS

(continued from p. 81)

- A normal probability curve is the familiar bell-shaped curve. If you graph the information in that curve as the cumulative probability, the result is an S-shaped curve. The 50% point on this curve is the peak on the original bell-shaped curve and represents 0 standard deviations. One standard deviation above is 84.13% and 15.83% is one standard deviation below. Standard deviations reported as cumulative percentages are probits if 5 is added to their value. The addition of 5 means that negative numbers are not considered.
- For the Core Experiment, if data are collected and solutions recorded as percentages, construct the graph using a log scale for the concentration.
- Brine shrimp are hypo-osmotic, that is, the concentration of water inside their bodies is greater than in the surrounding water. Water and salt are absorbed through their gut and the excess salt is removed by glands in their gills (Engemann & Hegner, 1981).
- The salinity of the Great Salt Lake is variable, but is usually between 17 to 28% (Allen, 1996).
- You can construct an alternate brine shrimp hatchery from a 2-L soda pop bottle as shown in Figure 3. Approximately 1700 mL of water fills this hatchery and the maximum amount of eggs is 4.0 g (1 teaspoon). When you want to collect hatchlings, turn off the air and pull up the air stone. About 10 minutes later, the hatchlings will have settled near the bottom and the eggs and egg shells will have floated to the top. You can draw off 500 mL of culture solution with concentrated brine shrimp by releasing the clamp. Approximately the first 20 mL or so that you draw off will include some abortive larvae and shell contamination. Draw off small samples and check them for undesirable contaminants before collecting some to use. Repeat this process on subsequent days until approximately 100 mL of culture remains in the inverted bottle.

there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each group of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 88.

TEACHER'S NOTES

Question

What is the effect of increased salinity on brine shrimp survival rate?

Hypothesis

Increasing the salinity will cause an increase in the survival rate of brine shrimp larvae.

Rationale

Brine is found in salt evaporation ponds and in the Great Salt Lake where salt concentrations are greater than that of the ocean.

Procedure

1. Prepare a series of NaCl dilutions ranging from distilled water at 0.0% NaCl to 4.0% NaCl with the 8.0% stock solution.
2. Label your dilution experiment containers with the percent NaCl as follows: 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625. Use 5.0 mL distilled water for the control at 0.0% NaCl.
3. Dispense 5.0 mL of distilled water into each container except the control.
4. Transfer 5.0 mL of the 8.0% solution to the distilled water in the container marked 4.0% and mix. Transfer 5.0 mL of the resulting 4.0% solution to 5.0 mL of distilled water in the container marked 2.0%. Continue this process until you have reached a dilution of 0.0625%. Discard 5.0 mL of the 0.0625% solution.

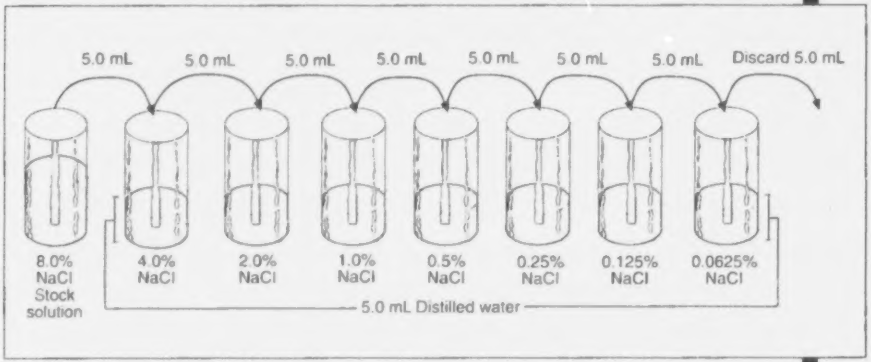


Figure 4. NaCl dilution series.

5. Dispense 0.5 mL of brine shrimp culture into each dilution.
6. Incubate the containers in light at room temperature for 24 hours.
7. Determine the mortality of brine shrimp in each NaCl concentration as follows:
   A. Make a copy of the template and tape it to the bottom of a petri dish. See Figures 5a & b. Use the template to keep track of the sample drops of brine shrimp that have been or remain to be counted.

TEACHER'S NOTES

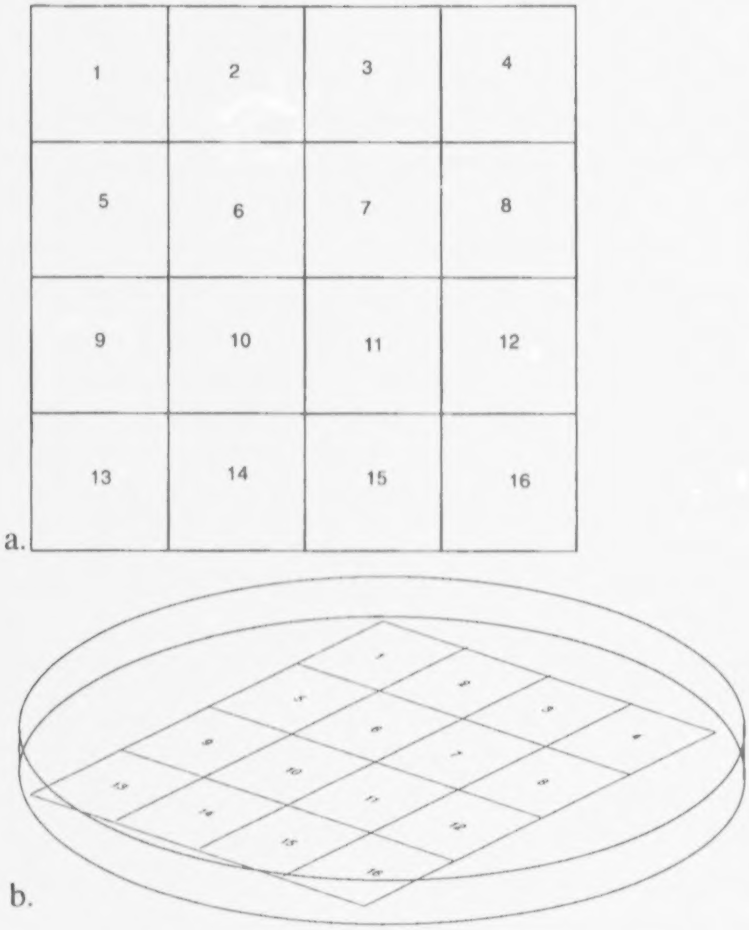


Figure 5 a. Petri dish template. b. Petri dish with template in place.

B. Stir the contents of the experimental container gently with the tip of a 1-mL pipette. Then, remove a small sample with the pipette.

C. Divide this sample into drops, putting one drop in each section of the grid in your petri dish. Vary the size of the drops depending on the activity and concentration of your shrimp. If there are many active shrimp, keep the drops small to count the shrimp as they swim.

D. Count and record the number of live and the number of dead brine shrimp in each sample. Stir the drop with a dissecting needle. Shrimp near the edge of the drop may not be visible. Stirring will bring them into view. Since you are calculating a percent mortality, you can stop counting when you have observed approximately 50 brine shrimp.

E. Calculate the percent mortality by dividing the number of dead brine shrimp by the total number of brine shrimp counted. If brine shrimp have died in the control, subtract the percent mortality of the control from the percent mortality of each treatment.

OR

8. If brine shrimp are purchased from a pet store, the following procedure can replace Steps 5 and 6.

A. Place brine shrimp population in a wide, open container, such as a culture dish.

B. Collect 10 brine shrimp and place in a 10-mL graduated cylinder.

C. Withdraw all but 1 mL of the water in the graduated cylinder. The 1 mL of water will contain a concentrated solution of brine shrimp. Dispose of the withdrawn water in a waste container.

D. Add the 1 mL of water containing the 10 brine shrimp to the first treatment container. To insure that all the brine shrimp are removed from the graduated cylinder, add 1 to 2 mL of the acid solution in the treatment container to the graduated cylinder. Swirl and extract the solution back into the original treatment container. **Be sure to start this procedure with the lowest acid concentration.**

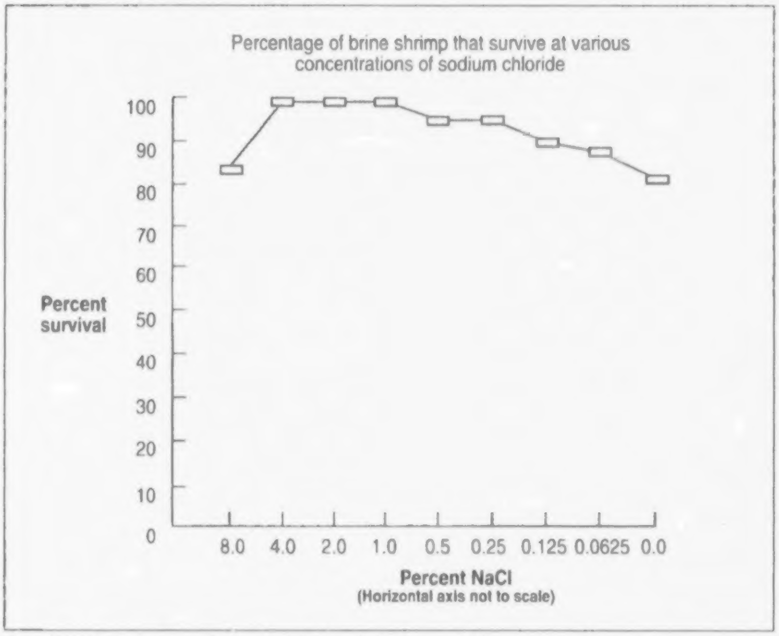
E. Repeat Steps 8 A to D for each treatment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 2. Survival of brine shrimp at various concentrations of sodium chloride.

Percent NaCl	Percent mortality
8.0	17.0
4.0	0.0
2.0	0.0
1.0	0.0
0.5	5.0
0.25	5.0
0.125	10.0
0.0625	12.0
0.0	18.0



Graph A. Salt concentration and brine shrimp survival.

TEACHER'S NOTES

Interpretation

Accept the hypothesis if more brine shrimp survive as the salinity increases. Reject the hypothesis if an increase in the salinity causes a decrease or no change in the survival rate.

Answer to Test Question

In a freshwater environment, the concentration of water outside the organisms would be much higher. Therefore, the osmotic potential would be increased greatly. Water would flow into the organism in larger amounts than normal. Most likely the organism would be incapable of removing this excess water.

TEST QUESTION

Brine shrimp live in a highly saline environment. Based on the results of this experiment, predict what would happen osmotically if brine shrimp were placed into a freshwater environment.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted their first experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- Was the survival of the brine shrimp the same at all NaCl concentrations?
- Was there a specific concentration that was the "best" or was there a range?
- Was there a point when the NaCl became harmful or toxic to the brine shrimp?
- To what other natural conditions or abiotic factors might brine shrimp be exposed? Could you design an experiment to find the optimal point for that organism's range of tolerance?
- To what environmental pollutants might brine shrimp be exposed? Can you test brine shrimp's tolerance to these factors?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

In this investigation, the participation of students who are visually impaired is limited where observations require the use of a microscope. These students might act as data recorders by recording these data with a braille writer or a tape recorder and providing a typewritten copy for the members of their lab groups.

- Provide labels in braille and in print for the containers.
- Increase the size of the object to be studied so they can use tactile means for observations.
- Consider substituting the larger freshwater crayfish for *Artemia*. The cost of saltwater crayfish would be likely prohibitive. In working with these animals, it is often advantageous to use small rubber bands to immobilize their claws. The investigation would now be to test the tolerance of crayfish for salt (NaCl) in the water. The size of the aquarium housing the crayfish is calculated to determine the appropriate amount of water to be used. The amount of NaCl to make 0.1% solution is measured and packaged. Additional packages of the same amount of salt can be prepared and stored to increase the salinity of the water. The animals should be observed for changes in behavior. Do not try to determine the percent of salt necessary to kill the crayfish. When there has been a drastic change in its actions, move the animal to a container of untreated water.

Gifted

These students may be challenged to determine an LD_{50} (lethal dose to 50% of the population) for a variation by fitting a line to the curve in the linear region or by performing a probit analysis. Although the statistical concepts involved in the probit analysis are complex, performing it with calculators that most students have is not difficult. Table 3 data are unlikely to result from the Core Experiment and are included only to illustrate the technique. Directions for this analysis involve making two transformations of these data: finding the best straight line through the transformed data, and finding out the value of the dose percentage when the mortality value is 50%. The first transformation is to convert dosages or percent acidity to the log of those values. A log transformation converts an exponential function to a linear function. The second transformation is to convert dosages in percent to probits. You make this transformation by using Table 3 from Finney (1971). The best straight line describing the transformed data can be found by using a calculator to find the regression line. Finally, substitute a probit value of 5 (50% mortality) in the equation and determine the dose percentage. Remember to convert by dose percentage that you obtain as a log to its antilog.

Table 3. Transformation of percentages to probits (from Finney, 1971. Reprinted with the permission of Cambridge University Press).

Percent	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.42	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.82	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Example:

Percent HCl dose	Log dose	Percent mortality	Mortality point
0.00001	-5.00	17	4.05
0.00005	-4.30	45	4.87
0.0001	-4.00	59	5.23
0.0005	-3.30	66	5.41
0.001	-3.00	90	6.28
0.005	-2.30	98	7.05
0.01	-2.00	100	—
0.05	-1.30	93	6.48
0.10	-1.00	100	—

TEACHER'S NOTES

TEACHER'S NOTES

Answers to Additional Test Questions

1. B
2. A scientist could raise brine shrimp in a solution with a slightly higher concentration of acid. After several days, the living brine shrimp could be removed and allowed to reproduce. Although all of the surviving brine shrimp are able to cope with a higher acid concentration, their mechanisms for survival may not be the same. The scientist could repeat this procedure, increasing the acidity slightly, for several generations; and each generation should be better able to live in an environment with a higher acid concentration. This demonstrates natural selection; individuals with beneficial characteristics will survive to pass these genetic traits on to their offspring.

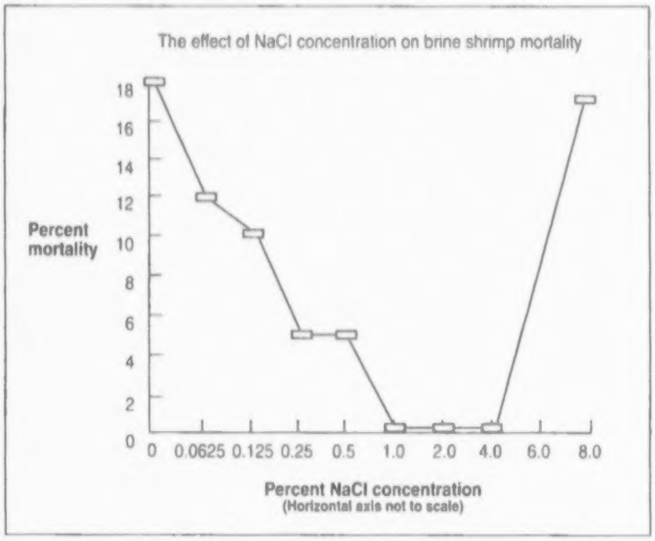
Mobility Impaired

- Review the selection of laboratory partners. A considerable amount of the time for this investigation is spent using a microscope. The instructor should check the access and comfort level of the student while using a microscope.
- Discuss the ability of the student who is mobility impaired to obtain supplies.
- Provide a microscope that can be operated by levers for students who are manually impaired. Sighted lab partners may assist with adjusting the field of view.
- Provide a tape recorder for taking notes for manually impaired students.
- Arrange for a lab partner to make the graphs of these data summarized by the student who is manually impaired.
- See "Allelopathy" for further considerations.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. According to the following graph, at what salt concentration did the brine shrimp have the highest survival rate?
   A. 8.0%
   B. 4.0%
   C. 0.5%
   D. 0.125%
   E. 0.0625%



Graph B. The effect of salt concentration on brine shrimp mortality.

2. Natural selection occurs when environmental conditions change and some individuals have a difference that allows them to survive and reproduce. When they reproduce, the offspring inherit the difference that allowed their parents to survive. Using brine shrimp, how could a scientist illustrate the process of natural selection?

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VARIATIONS OF THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

VARIATION 1

The Effect of Increased Alkalinity on Brine Shrimp

SYNOPSIS

Students will determine if an increase in the concentration of the base NaOH will affect survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 10 mL 1.0% NaOH solution

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Follow the same procedure as in the Core Experiment substituting sodium hydroxide for the NaCl.
- Dissolve 1.0 g of NaOH in 100 mL of 1.0% NaCl solution.

Answers to Questions and Analysis on Student Page

1. These laboratory data correlate with the hypothesis that if salt concentration increases above 4.0%, then the survival rate decreases.
2. Answers will vary. Examples of possibilities are miscounting, poor laboratory skills, old brine shrimp eggs, unknown impurities in the water such as fluorine.
3. Practice laboratory procedures such as using a pipette, syringe, or graduated cylinder before conducting the actual experiment. Repeat your experiment looking for discrepancies. Use large sample sizes and average your results. Check all of your math work for errors.
4. The graph shows that brine shrimp have an optimum tolerance level for salt. While a few will survive in a low concentration of salt, those shrimp in less concentrated solutions had the lowest survival rate.
5. Answers will vary. Examples could be improved laboratory skills and math skills, a greater understanding of the scientific inquiry process, and appreciation of the delicate balance that exists in nature.
6. Answers will vary depending on the topic you are studying. You could relate this laboratory to studies of ecology, marine biology, osmo-regulation, tolerance, and cell biology.
7. The laboratory results indicated that brine shrimp have an optimum tolerance level for salt. Also, while brine shrimp survival is reduced at certain concentrations, we do not know if similar results would occur in other organisms. These pose questions that could serve as topics for further investigation.
8. This laboratory uses an understanding of many areas of science. In order to appreciate fully and understand the laboratory, one needs a knowledge of chemistry, ions, chemical formulas, membrane transport, ecology, and marine systems.

TEACHER'S NOTES

TEACHING TIPS

Seawater and the Great Salt Lake are alkaline. Allen (1996) found increased survival of brine shrimp if he used sodium carbonate to increase the pH value to 8.5.

HYPOTHESIS GENERATION

Question

How will increasing the pH of the water environment, i.e., making it more basic, affect brine shrimp?

Sample Hypothesis

Increasing the concentration of the base NaOH will cause an increase in the mortality rate of brine shrimp larvae.

Rationale

Animal tissues dissolve in bases. Increased concentration of a base should result in increased physiological stress and increased mortality.

Sample Experimental Procedure

1. Label the dilution experimental containers to represent the desired final concentration of NaOH. Include 1 container with 1.0% NaCl, but no NaOH.
2. Prepare several concentrations of NaOH as follows:
   A. Dispense 9 mL of 1.0% NaCl into each of the labeled containers.
   B. Add 1 mL of 1.0% NaOH to the dilution container labeled 0.1%. Mix thoroughly.
   C. Use the same pipette to transfer 1 mL of the 0.1% solution to the dilution container labeled 0.01%.
   D. Use the same pipette to repeat the process of Steps A to C to create your additional dilutions. See Figure 6.

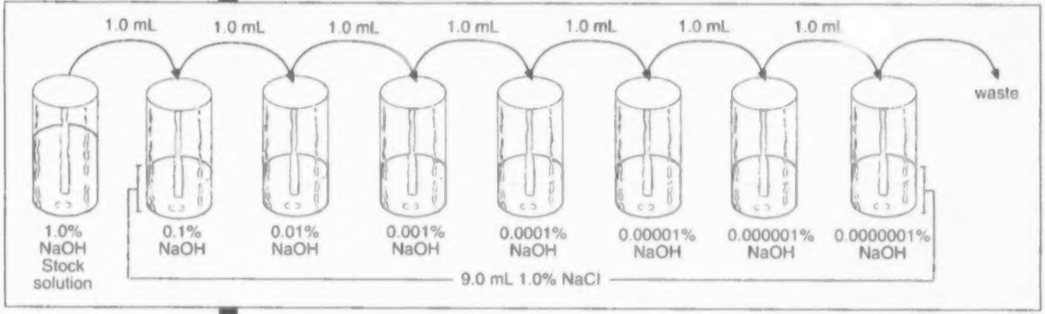


Figure 6. Serial dilution of NaOH.

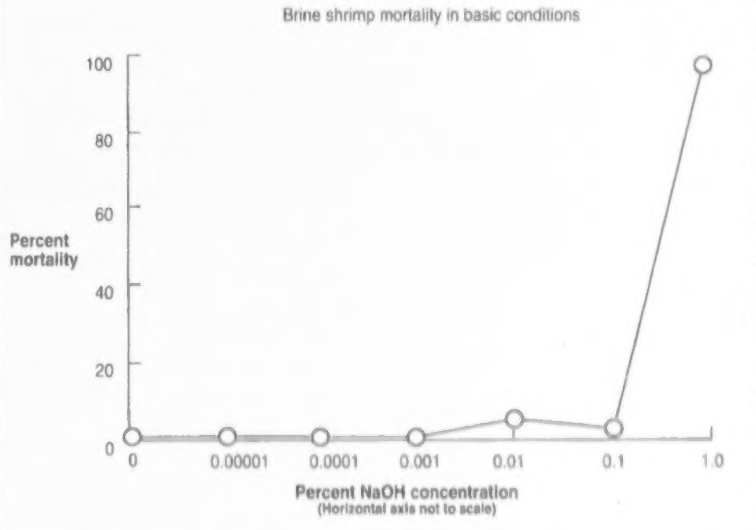
   E. Remove and discard 1 mL from the weakest solution.
3. Dispense 1 mL of brine shrimp with a plastic, disposable pipette into each container.
4. Incubate the containers in light at room temperature for 24 hours.
5. Follow the procedure of the Core Experiment to determine the mortality of brine shrimp in each basic concentration.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 4. Brine shrimp mortality in basic conditions.

Percent NaOH	Number of live brine shrimp	Number of dead brine shrimp	Percent mortality
0.0	50	0	0
0.00001	50	0	0
0.0001	50	0	0
0.001	50	0	0
0.01	48	2	4
0.1	49	1	2
1.0	0	50	100



Graph C. NaOH effect on brine shrimp mortality.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Design investigations similar to those using brine shrimp.
- Have students who are blind observe crayfish manually and by listening to the sounds as the animals move through the water.
- Make a raised-line drawing or a braille painting of the brine shrimp and the crayfish if models are not available in the following ways:

Raised-line sketch. Sketch a cross section of the brine shrimp and of the crayfish on braille paper. Place the sketch on a rubber mat or on a wooden board covered with several layers of heavy cloth. Trace the sketch with a variety of tools that give various sizes and depths of lines. Use a stapling tool to give variety to the surface texture or a sewing tracing wheel to give the feel of a dotted line.

Braille painting. Draw the cross section of the animal desired on canvas board or heavy cardboard. Use Elmer's Glue® to make a narrow raised line covering the

TEACHER'S NOTES

Interpretation

Accept the hypothesis if fewer brine shrimp survived as the concentration of NaOH increased. Reject the hypothesis if there was no change in the survival rate as the concentration of the NaOH increased. Also, reject the hypothesis if the survival rate peaks at a certain pH then decreases as the pH changes (either higher or lower) from that level. This suggests there is an optimal pH value.

pencil marks. Obtain variety by placing string, wire, rope, and other items in the glue. Sprinkle rice or sand in an area of glue to designate other tissue. There are no rules for making these 'paintings.' Just be creative. Some students gladly do these. Others do them to help a friend or to try something different. They are done in a thin cardboard box with a lid and can be stored for years.

VARIATION 2

The Effect of Thermal Pollution on *Artemia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will determine the optimal temperature for survival of *Artemia* larvae.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 1 thermometer (°C)
- 6 beakers
- 6 Styrofoam™ cup insulators or wide mouth thermoses
- 1 ice bath
- 1 hot water bath

HYPOTHESIS GENERATION

Question

What is the effect of temperature on brine shrimp survival

Sample Hypothesis

Survival will increase with increasing temperature up to a moderate temperature and then decline as the temperature is further increased.

Rationale

Survival depends on several enzyme systems working. In general, enzyme activity is slowed by cold and stopped by high temperatures. Optimal enzyme activity occurs at a moderate temperature or at a temperature close to the normal temperature of the organism.

Sample Experimental Procedure

1. Place 5 mL of a 1.0% NaCl solution into each of your sample vials.
2. Adjust the water in thermostatically controlled water baths or in vacuum bottles to the desired temperatures.
3. Balance or float one of your sample vials in each water bath for about 2 minutes to allow the salt solution to come to the desired temperature.
4. Add 0.5 mL of a stock brine shrimp solution to each jar. Monitor and maintain the temperature for at least 10 minutes by adding warm or cold water to the water bath.
5. Allow the water sample vials to return slowly to room temperature and maintain them at this temperature for 24 hours.
6. Follow the procedure of the Core Experiment to determine percent mortality.

TEACHER'S NOTES

TEACHING TIPS

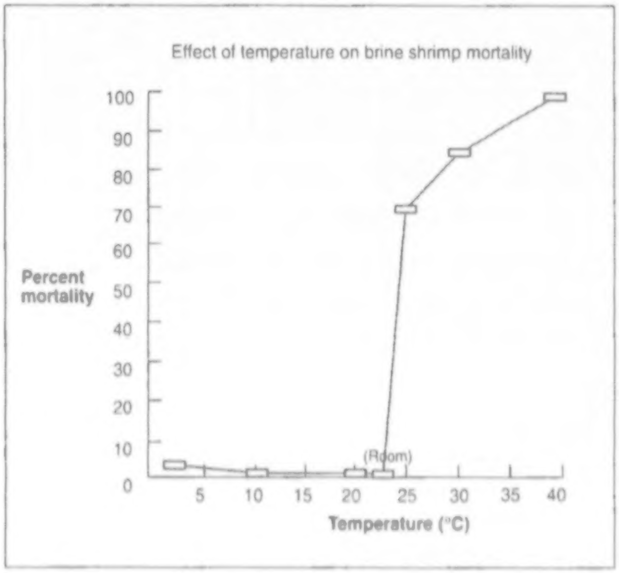
- Use temperatures of 2, 10, 20, 25, 30, 40°C.
- You can create Styrofoam™ collars from food trays to float sample vials.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Percent mortality of brine shrimp surviving after exposure to various temperatures for 10 minutes.

Incubation temperature (°C)	Percent mortality of brine shrimp
2	4
10	2
20	0
22 (Room)	0
25	70
30	86
40	100



Graph D. Relationship of temperature to brine shrimp mortality.

TEST QUESTION

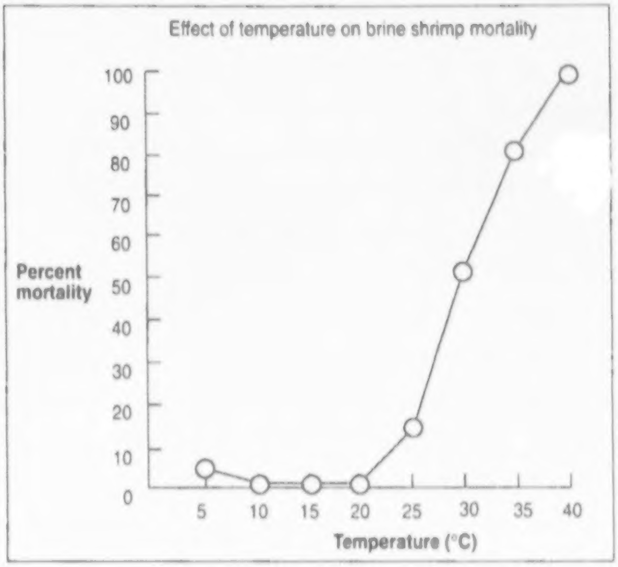
Graph E displays slightly different data from another group of students who conducted the same experiment as the students in Graph D. According to this set of data, what is the optimal temperature or range of temperatures for brine shrimp survival? How can you account for the difference in survival at different temperatures?

Interpretation

The hypothesis should be accepted if the greatest number of living brine shrimp are counted at an intermediate temperature. Reject the hypothesis if the highest survival occurs at extreme temperatures.

Answer to Test Question

Brine shrimp survival is optimum where mortality is minimal. This occurs over the tested temperature range of 10 to 20°C. Temperatures lower than 10°C may be too cool to provide the necessary heat for molecular interactions, while temperatures greater than 20°C may alter the shape of the enzymes necessary for life-sustaining metabolic reactions.



Graph E. Brine shrimp mortality.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 3

Determination of Preferred Depth of Migration of Artemia

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will determine if the larvae of *Artemia* show a preference for a specific depth.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 1 2-m piece of Tygon™ tubing
- 42 plastic pinch or screw clamps
- 1 meter stick
- 1 pair of scissors
- 40 67-mL (2-oz) jars, small beakers, or vials

HYPOTHESIS GENERATION

Question

Will *Artemia* show a preference for shallow depths?

Sample Hypothesis

The larvae of *Artemia* will show a preference for shallow depths.

Rationale

Brine shrimp larvae feed on algae that require sunlight to photosynthesize. Consequently the brine shrimp will prefer shallow depths.

TEACHER'S NOTES

TEACHING TIPS

- Brine shrimp may show diurnal rhythms independent of the light or dark period you provide. Many planktonic organisms migrate diurnally, following their food source. The rhythm continues in continuous dark.
- If brine shrimp depth preference is related to their choice of a particular light intensity, you should get different results in a tube continuously lighted and one wrapped in dark paper or foil and lighted only from the top. However, light intensity provided by classroom fluorescent lights may not be adequate to show a difference.

SAMPLE EXPERIMENTAL PROCEDURE

1. Close off the bottom of a 2-m piece of Tygon™ with a clamp or a rubber stopper.
2. Use a meter stick to position additional clamps onto the tubing at 5-cm intervals with enough pressure so that they will not slide, but not tight enough to seal the tubing. See Figure 7.

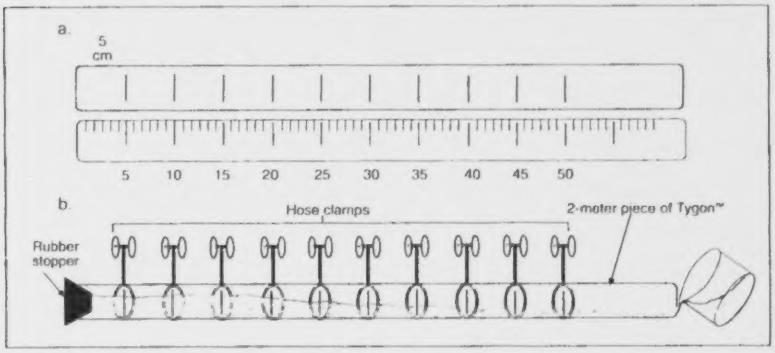


Figure 7. Position clamps every 5 centimeters.

3. While holding the tubing parallel to the floor, fill it with brine shrimp solution until it almost reaches the top of the tube.
4. Hang the tube upright.
5. Allow brine shrimp to migrate for several hours.
6. Without disturbing the location of brine shrimp, seal off the clamps one at a time beginning at the bottom.
7. Position a labeled collection container under the tube and remove the stopper or closing clamp. See Figure 8.

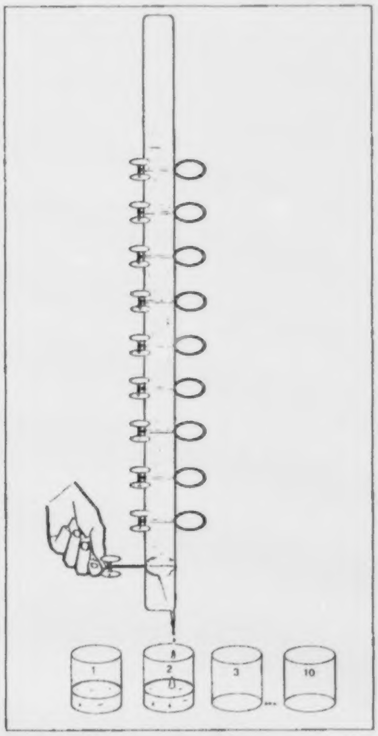


Figure 8. Tygon™ in vertical position with collection chamber.

TEACHER'S NOTES

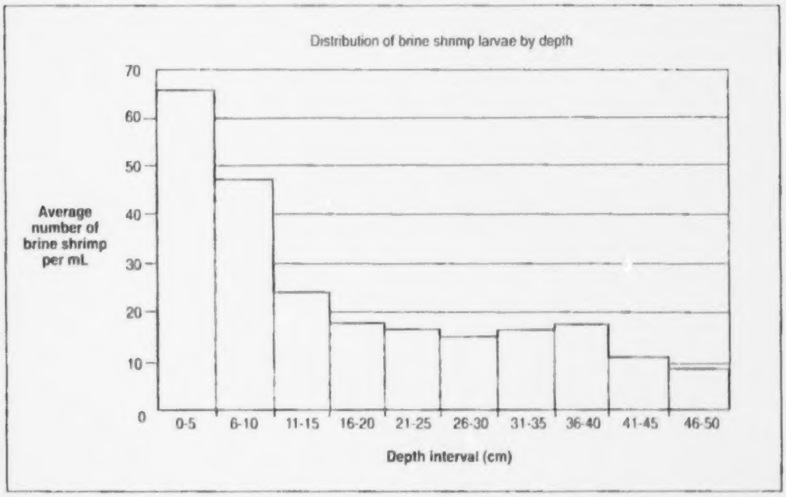
8. Repeat the process for each section until the tube is emptied.
9. Count the number of living brine shrimp in a measured volume from each section. Use the drop counting technique of the Core Experiment, but vary the volume that you count. Where 1 mL contains several hundred brine shrimp, a 0.25 mL sample will be enough, but where the density is very low, you may need to count more milliliters to find 50 organisms.
10. Adjust all counts to per milliliter. For example, if you have counted only 0.25 mL, multiply your value by 4. If you have counted 2 mL, divide your value by 2.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Distribution of brine shrimp larvae by depth in a narrow column of salt water.

Depth interval (cm)	Average number of live brine shrimp (per mL)
0 to 5	68
6 to 10	48
11 to 15	24
16 to 20	16
21 to 25	14
26 to 30	13
31 to 35	14
36 to 40	16
41 to 45	11
46 to 50	9

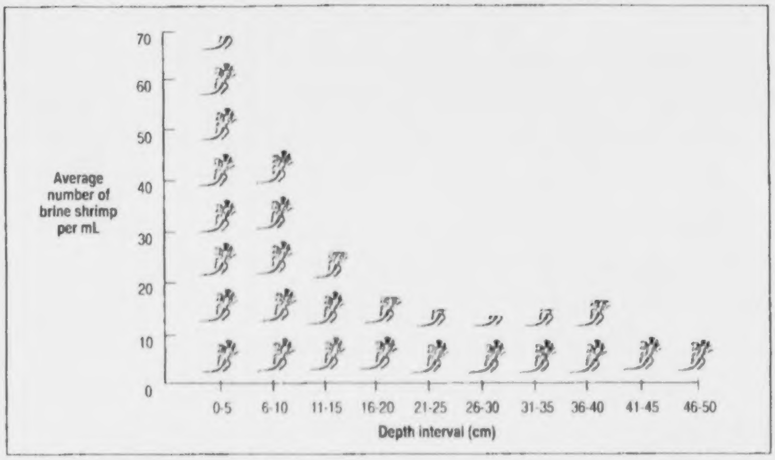


Graph F. Column graph showing the distribution of brine shrimp larvae by depth.

Alternatively, you can display these data in a pictograph such as the one shown in Graph G.

Interpretation

Accept the hypothesis if more brine shrimp were counted in the sections of tube nearest to the surface of the water. Reject the hypothesis if there was an even distribution of the brine shrimp throughout the length of the tubing or if more individuals were counted at lower depths.



Graph G. A pictograph showing the distribution of brine shrimp larvae by depth.

TEST QUESTION

Life exists at various depths in the aquatic environment. In this experiment, where were the majority of brine shrimp found? Explain why they tend to stay at this depth.

Answer to Test Question

They are filter feeders who primarily eat autotrophic phytoplankton that should be located near the surface where there is enough light for them to photosynthesize.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

The topic of 'determining the depth of migration of brine shrimp' is not practical to do with a crayfish. The blind student might investigate the activity of crayfish in the dark and various amounts of light.

VARIATION 4

The Effect of Increased Sodium Phosphate Concentration on Brine Shrimp

***Note to Teachers:** In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.*

SYNOPSIS

Students will determine if an increase in sodium phosphate concentration will affect the survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 10 mL 8.0% sodium orthophosphate ($Na_3PO_4 \bullet 12H_2O$)

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Follow the same procedure as in the Core Experiment substituting a series of solutions from 0.0% to 8.0% of sodium phosphate. To prepare 8.0% sodium phosphate, dissolve 8.0 g of sodium phosphate in a small amount of distilled water, then dilute it to 100 mL with distilled water. Sodium phosphate makes available sodium, so **do not use 1.0% NaCl as the diluent.**

HYPOTHESIS GENERATION

Question

Will increasing phosphate concentration affect the survival rate of brine shrimp?

Sample Hypothesis

Increasing the phosphate concentration will cause a decrease in the survival of brine shrimp larvae.

Rationale

Phosphates have been eliminated from most cleaning products because of their damage to the environment so phosphate may harm organisms directly.

Sample Experimental Procedure

1. Label the vials with the following Na_3PO_4 dilutions: 8.0%, 4.0%, 2.0%, 1.0%, 0.5% and 0.25%.
2. Dispense 5 mL of distilled water into all the treatment vials except the one reserved for 8.0% sodium phosphate.
3. Transfer 5 mL of the 8.0% solution into the 4.0% vial. Mix well and remove 5 mL.
4. Transfer the 5 mL you removed from the 4.0% vial to the vial labeled 2.0%. Mix this well and remove 5 mL.
5. Continue the transfer-mix process until you have made all the desired dilutions.
6. Remove 5 mL from the last dilution so that the volume is the same as in your other vials.
7. Add 0.5 mL of culture to each dilution.
8. After 24 hours, count and classify the organisms as lively, barely alive, or dead using the drop counting technique of the Core Experiment.

TEACHING TIP

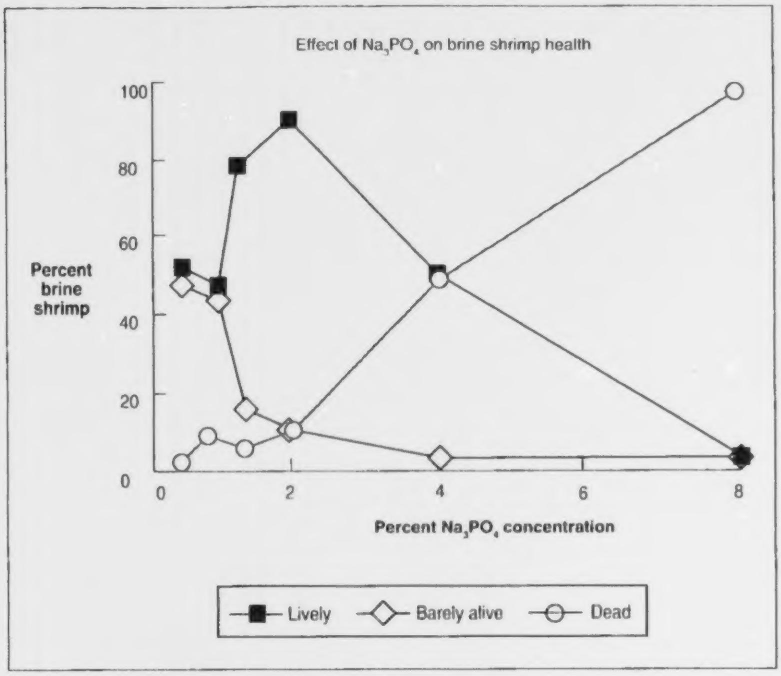
The hypothesis is written for testing various concentrations of sodium phosphate. If your students want to compare the effect of phosphate separate from the effect of sodium, they should calculate the solutions in equivalents. An 8.0% sodium orthophosphate solution is 0.17N and offers three times the amount of sodium as a 0.17N sodium chloride solution. They will want to have a separate sodium chloride control for each sodium orthophosphate dilution. For the 8.0% sodium orthophosphate solution, the control would be a 2.98% sodium chloride solution. When doing their calculations, they will subtract the mortality in the matching control from the mortality in the treatment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 7. Effect of sodium phosphate on apparent brine shrimp health.

Phosphate concentration (%)	Number lively	Number barely alive	Number dead	Percent lively	Percent barely alive	Percent dead
8.0	0	0	50	0	0	100
4.0	0	34	33	0	51	49
2.0	3	45	3	6	88	6
1.0	4	61	3	16	80	4
0.5	34	35	5	46	47	7
0.25	42	43	0	49	51	0



Graph H. Graphical display of apparent health of brine shrimp after 24 hours exposure to various concentrations of sodium orthophosphate.

TEST QUESTION

In a natural system, brine shrimp eat photosynthetic algae that they filter from the water. How might the concentration effects of phosphate be different if algae were present in your experimental system? Consider both the short term 1 to 5 days and long term 1 to 2 months effects.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 5

The Effect of Nitrate Concentration on *Artemia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Students will determine if an increase in nitrate concentration will affect survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 10 mL 10.0% $NaNO_3$

Interpretation

Accept the hypothesis if a decrease in the survival rate is observed whn there is an increase in the phosphate concentration. Reject the hypothesis if there is no change in the survival rate as the concentration of phosphate increases or if the survival mrate decreases as the phosphate concentration decreases.

Answer to Test Question

If algae were present and the systems were in the light, one would expect the algae to take up phosphate from the solution. That would reduce quickly the concentration making a given addition of phosphate less toxic to the brine shrimp. One would expect the low concentrations of phosphate then to enhance the growth of algae. More algae would mean more food for the brine shrimp, resulting in greater survival and reproduction. Over a still greater time with high phosphate concentrations, algae should grow so well that as they die they create a large source of food for bacteria. The bacterial growth then will reduce the availability of oxygen and result in the asphyxiation of the brine shrimp.

TEACHING TIP

The hypothesis is written for testing various concentrations of sodium phosphate. If your students want to compare the effect of phosphate separate from the effect of sodium, they should calculate the solutions in equivalents. An 8.0% sodium orthophosphate solution is 0.17N and offers three times the amount of sodium as a 0.17N sodium chloride solution. They will want to have a separate sodium chloride control for each sodium orthophosphate dilution. For the 8.0% sodium orthophosphate solution, the control would be a 2.98% sodium chloride solution. When doing their calculations, they will subtract the mortality in the matching control from the mortality in the treatment.

Interpretation

The hypothesis should be accepted if a decrease in the survival rate is observed when the concentration of nitrate increases. The hypothesis should be rejected if there is no change in the survival rate as the concentration of nitrate increases or if the survival rate decreases as the nitrate concentration decreases.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Follow the same procedure as in the Core Experiment substituting a series of sodium nitrate solutions from 0.0% to 8.0%. To prepare an 8.0% sodium nitrate solution, dissolve 8.0 g of sodium nitrate in a small amount of distilled water and dilute to 100 mL with distilled water. Sodium nitrate makes sodium ions available, so **do not use 1.0% NaCl as the diluent.**

HYPOTHESIS GENERATION

Question

What effect will the increase in nitrate concentrations have on brine shrimp?

Sample Hypothesis

An increase in the concentration of nitrate will cause a decrease in the survival of brine shrimp larvae.

Rationale

Nitrate pollution is viewed as a serious problem resulting from wide use of fertilizers in agriculture and horticulture.

Sample Experimental Procedure

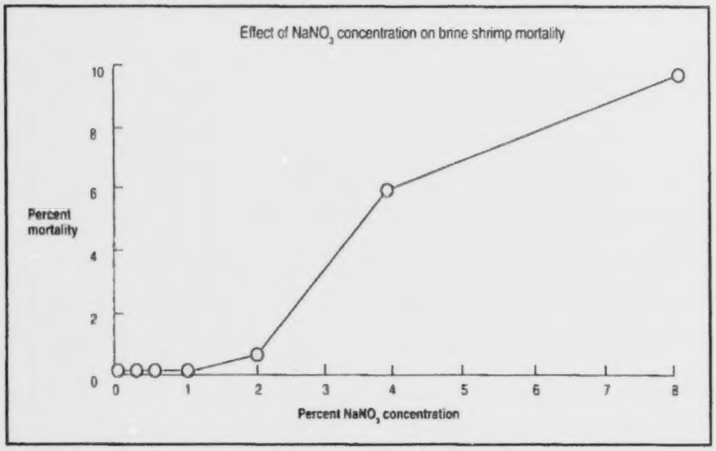
1. Repeat Steps 1 to 7 from Variation 4, substituting sodium nitrate for sodium phosphate.
2. After 24 hours, count and classify the organisms using the drop counting technique of the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 8. Mortality of brine shrimp in various sodium nitrate solutions.

Percent $NaNO_3$ concentration	Percent mortality
8.0	100
4.0	58
2.0	5
1.0	0
0.5	0
0.25	0
0.125	0
0.0625	0



Graph I. Effect of nitrate concentrations on brine shrimp mortality.

TEST QUESTION

Most commercial fertilizers contain nitrates. As rain falls on an agricultural field, the runoff may dissolve the nitrates in the fertilizers and carry them into the groundwater that may eventually reach rivers and oceans. What might this do to organisms living in the water?

Answer to Test Question

In the same way that nitrates fertilize crops, they act as fertilizers to algae and plants in the water. They stimulate their growth and the population increases. As the plants increase in size and number, they use oxygen. The plants compete with the animal life for oxygen. Ecosystems can be threatened seriously by increased levels of nitrates.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 6

The Effect of Substituting a Different Metal Ion for the Sodium Ion in the Culture Solution on the Survival of the Larvae of Brine Shrimp

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will determine if replacing the sodium ion of NaCl will affect survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 1.0% solution of any of these possible salts: KCl, $CaCl_2$, $NiCl_2$, $FeCl_3 \bullet H_2O$, $CuCl_2$

HYPOTHESIS GENERATION

Question

What effect do other metal ions have on brine shrimp larvae survival?

Sample Hypothesis

A substitution for the sodium ion in the culture solution will cause a decrease in the survival rate of the brine shrimp larvae.

Rationale

Brine shrimp would be unlikely to have protection from high levels of other metal ions because they would not have experienced them during their evolution.

Sample Experimental Procedure

1. Repeat Steps 1 to 7 from Variation 4, substituting the appropriate salt concentrations from 0.0625 to 1.0% only for each salt.
2. After 24 hours, count and classify the organisms using the drop counting technique of the Core Experiment.

TEACHING TIPS

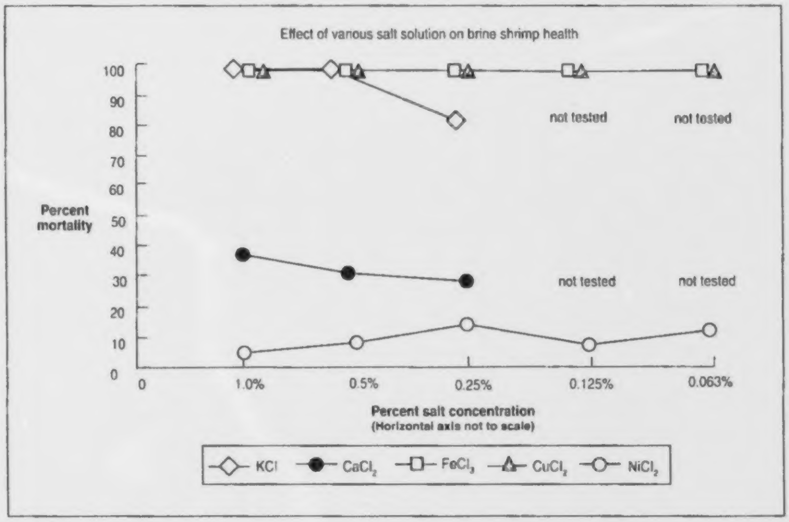
- Concern over the damaging effects of sodium chloride used to de-ice roads in winter has led to the replacement of sodium with other metal ions. Students might be interested in discovering what is used in your area or in an area where they have family or where they vacation. Substituting this material for the shelf chemicals would be a relevant variation.
- The appropriate controls are sodium chloride concentrations equivalent to the chloride ion concentrations provided by the test salt solutions. Over most of the range of concentrations students will not find depressed mortality due to chloride ions. Coordinate with a group planning to study the effect of sodium chloride concentrations as in the Core Experiment, or through a coordinated dilution scheme.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 9. Average brine shrimp mortality (%) in various salt solutions of several concentrations corrected for mortality in sodium chloride.

Salt solution	1.0%	0.5%	0.25%	0.125%	0.0625%
KCl	100	100	86	Not tested	Not tested
$CaCl_2$	37	30	26	Not tested	Not tested
$FeCl_3$	100	100	100	100	100
$CuCl_2$	100	100	100	100	100
$NiCl_2$	4	5	8	4	7



Graph J. Effect of metal ion substitution on brine shrimp mortality.

TEST QUESTION

Normally brine shrimp live in a NaCl solution. What role does the sodium ion play in the survival of organisms? What would happen if you substituted calcium chloride for sodium chloride?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

Interpretation

The hypothesis needs to be tested and either accepted or rejected separately for each ion substitution made. Accept the hypothesis if the substitution of an ion causes a decrease in the survival rate. Reject the hypothesis if the ion substitution causes an increase or no change in the survival rate.

Answer to Test Question

If the sole purpose of sodium ions is to maintain the osmotic potential, changing the metal ion would have little effect. The results of the experiment suggest that sodium has additional functions in the survival of brine shrimp. It is possible that brine shrimp rely on a sodium-potassium pump mechanism for osmotic regulation.

VARIATION 7

The Effect of Substituting a Different Halogen Anion for the Chloride Ion on the Survival of Brine Shrimp Larvae

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students will determine if replacing the ion in NaCl will affect survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 1.0% solution of any of these possible salts: NaI, NaBr, NaF

HYPOTHESIS GENERATION

Question

What effect will the substitution of a halogen ion have on the survival rate of brine shrimp?

Sample Hypothesis

A substitution for the chloride ion in the culture solution will cause a decrease in the survival rate of the brine shrimp larvae.

Rationale

The concentration of bromine in the ocean is 65 ppm with fluorine at 1.4 ppm and iodine at 0.05 ppm. All are very small when compared to chlorine at 18,980 ppm (Hodgman, Weast, & Selby, 1961). You would not expect brine shrimp that have had little contact with high concentrations of halogens, other than chlorine, throughout their evolution to have established high tolerances for them.

Sample Experimental Procedure

1. Repeat Steps 1 to 7 from Variation 4, substituting the appropriate concentrations of the halogen from 0.032 to 1.0%.
2. After 24 hours, count and classify the organisms using the drop counting technique of the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 10. Average brine shrimp mortality (%) in various salt solutions of several concentrations.

Salt solution	1.0%	0.5%	0.25%	0.125%	0.0625%	0.032%
NaF	100	100	100	100	100	Not tested
NaBr	70	40	10	8	8	30
NaI	56	55	60	55	61	77

TEACHER'S NOTES

TEACHING TIPS

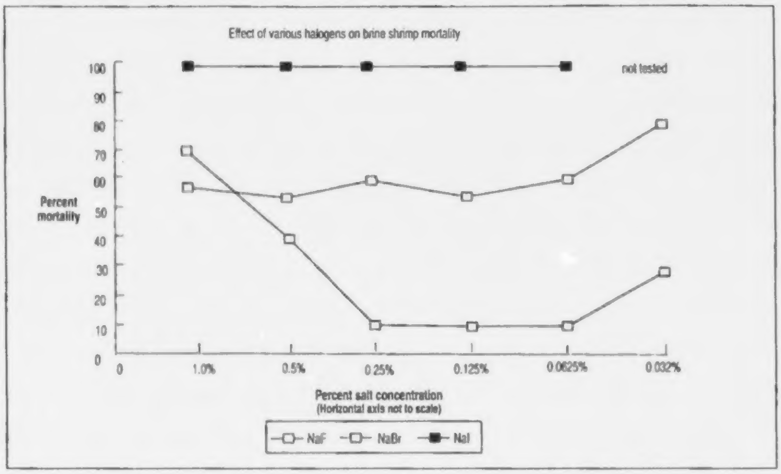
- The appropriate control is a sodium chloride concentration equivalent to the sodium ion concentration provided by the test salt solution. Over the range of salt concentrations tested here, most students would have found 100% survival of brine shrimp larvae. Coordinate with a group planning to study the effect of sodium chloride concentrations as in the Core Experiment, or through a coordinated dilution scheme.
- If your students are capable of constructing an experiment as complex as Variation 7, consider encouraging someone to test baking soda here, although the bicarbonate anion (HCO_3^{-1}) is a complex ion rather than a halogen anion.

Interpretation

The hypothesis needs to be tested and either accepted or rejected separately for each ion substitution made. Accept the hypothesis if the substitution of an ion causes a decrease in the survival rate. Reject the hypothesis if the ion substitution causes an increase or no change in the survival rate.

Answer to Test Question

If one used iodized table salt to prepare the culture salt solution for brine shrimp, one would expect to have a very poor harvest. If sodium iodide was used instead of sodium chloride, the harvest would be even worse because iodide ions would be more concentrated.



Graph K. The relationship of salt concentration to brine shrimp survival.

TEST QUESTION

Normally the brine shrimp live in a NaCl solution. What would happen if you substituted sodium iodide for sodium chloride or if you prepared brine shrimp culture solutions using iodized table salt?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 8

The Effect of Combining a Harmful Cation with an Apparently Safe Anion on Brine Shrimp Survival

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will determine how the exposure of *Artemia* to the combination of a 0.125% $FeCl_3 \bullet 6H_2O$ solution and a 0.125% $NaHCO_3$ solution will affect survival of the larvae.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 10 mL of 1.0% $FeCl_3 \bullet 6H_2O$
- 10 mL of 1.0% $NaHCO_3$

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Prepare a 1.0% solution of $FeCl_3$ by dissolving 1.0 g of $FeCl_3$ in a small amount of distilled water and diluting the resulting solution to 100 mL with distilled water.
- Prepare a 1.0% solution of sodium hydrogen carbonate (baking soda) by dissolving 1.0 g of $NaHCO_3$ in a small amount of distilled water and diluting the resulting solution to 100 mL with distilled water.

HYPOTHESIS GENERATION

Question

What effect will combining a harmful cation with a "safe" anion have on brine shrimp survival?

Sample Hypothesis

The addition of $NaHCO_3$ to a $FeCl_3$ solution will cause an increase in the survival of brine shrimp larvae.

Rationale

Students should provide their own rationale.

Sample Experimental Procedure

1. Label your vials for a dilution series producing the desired dilutions. If you want to test the effect of 0.125% $NaHCO_3$ and 0.125% $FeCl_3$ you will need that concentration of each solution and a 0.25% solution of each compound. If you combine 2.5 mL of 0.25% of $NaHCO_3$ with 2.5 mL of 0.25% $FeCl_3$, the resulting solution will be 0.125% in each compound.
2. Label experimental containers: Na_3Cl control, $NaHCO_3$, $FeCl_3$, and $NaHCO_3$ + $FeCl_3$.
3. Dispense 10 mL of distilled water into all the dilution containers.
4. Into the 0.5% vial, transfer 10 mL of the 1.0% solution. Mix this well and remove 10 mL.
5. Transfer the 10 mL you removed from the 0.5% vial to the vial labeled 0.25%. Mix this well and remove 10 mL.
6. Continue the transfer-mix process once more to create 10 mL of 0.125%.
7. Transfer 5 mL of the final dilution to the vials labeled for a single compound. Transfer 2.5 mL of the next to the last dilution of each solution to the vial labeled for both solutions.
8. Put 5 mL of 1.0% NaCl in the NaCl control container.
9. Add 0.5 mL of culture to each experimental container.
10. After 24 hours, count and classify the brine shrimp larvae using a slight modification of the drop counting technique of the Core Experiment. Here count exactly 50 brine shrimp from each solution and record the percent of survivors.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 11. Survivorship of brine shrimp larvae in various solutions of $FeCl_3$ and $NaHCO_3$. The solution that has no iron or bicarbonate is 1.0% NaCl.

Solution	0.0% $FeCl_3$	0.125% $FeCl_3$
0.0% $NaHCO_3$	50	0
0.125% $NaHCO_3$	47	44

Table 12. Survivorship percentage of brine shrimp larvae in various solutions of $FeCl_3$ and $NaHCO_3$.

Solution	0.0% $FeCl_3$	0.125% $FeCl_3$
0.0% $NaHCO_3$	100	0
0.125% $NaHCO_3$	94	88

Display the percent survivors for each treatment plotted against the treatment on the x-axis.

TEACHING TIPS

- Have students mix a drop of the 1.0% solutions together to observe what happens. The precipitate that forms will resemble tomato juice. They will see that it would be difficult to count larvae in such a solution. Encourage them to consider more dilute solutions.
- The reaction that occurs when the solutions are mixed reduces the concentration of iron ions:

  $2FeCl_3 + 2NaHCO_3 \rightarrow$
  $Fe_2(CO_3)_3\downarrow +$
  $2NaCl + CO_2\uparrow + H_2O$

  To clean up, students can precipitate a pollutant and remove the precipitate.
- Students may want to consider whether these solutions should be made in 1.0% NaCl and all dilutions made using 1.0% NaCl. The directions are written with only distilled water, but you easily can substitute 1.0% NaCl for distilled water.

Interpretation

Accept the hypothesis only if both the $FeCl_3$ solution causes a decrease in the survival rate and the addition of $NaHCO_3$ to the $FeCl_3$ solution reduces its negative effects. Reject the hypothesis if the $FeCl_3$ did not cause a decrease in the survival rate. Also reject the hypothesis if the $FeCl_3$ caused a decrease in the survival rate but the $NaHCO_3$ did not reduce the negative effects of the $FeCl_3$.

TEST QUESTION

Iron can be leached from the soil by acid rain thereby releasing Fe ions into the water. Iron ions greatly reduce survivorship of brine shrimp larvae. Adding sodium bicarbonate reduces the effects of the iron. How would you explain this?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 9

The Effect of a Simulated Oil Spill on Brine Shrimp Larvae

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS

Students will compare mortality of brine shrimp when exposed to increasing amounts of motor oil.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 1 mL new motor oil
- 1 fine-tipped dropping pipette

Table 13. Calculation of percent surface area covered in drops.

Diameter (cm) covered	Percent covered	Number of oil drops
0.8 cm	20%	2 drops
1.6 cm	40%	10 drops
2.4 cm	60%	41 drops
3.2 cm	80%	52 drops
4.0 cm	100%	55 drops

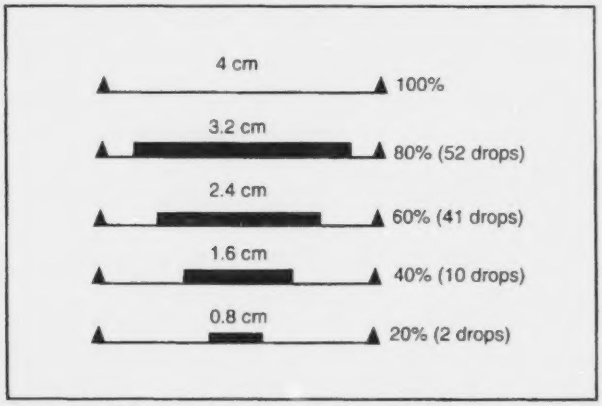


Figure 9. Side view of area covered with number of oil drops indicated in Table 13. Scaled to the 4-cm diameter of a film canister.

Answer to Test Question

There will be many answers to this question and you should be more concerned with the thought process rather than the "correct" answer. The most likely explanation is the sodium bicarbonate chemically ties up the Fe ions, thereby removing them from the solution.

TEACHING TIPS

- Film cans work especially well for this variation because the surface area is larger than in a test tube, but not so large as to require a lot of oil to cover the surface.
- Have the students draw several circles the same size as the film can and fill in 20%, 40%, 60%, and 80% of the surface so that they have a model for what they expect when they adequately have covered the surface for each treatment.
- Alternatively, have students count the number of drops to determine these percentages. Table 13 shows a representative set of number of drops in relation to the percent surface area covered. See Figure 9 for a graphic representation of this information.

HYPOTHESIS GENERATION

Question

What effect will increasing the concentration of oil have on brine shrimp survival?

Sample Hypothesis

Increasing the amount of oil on the surface of water will increase the mortality of brine shrimp larvae.

Rationale

The oil will form a surface over the water preventing the access to aeration that the brine shrimp need to survive.

Sample Experimental Procedure

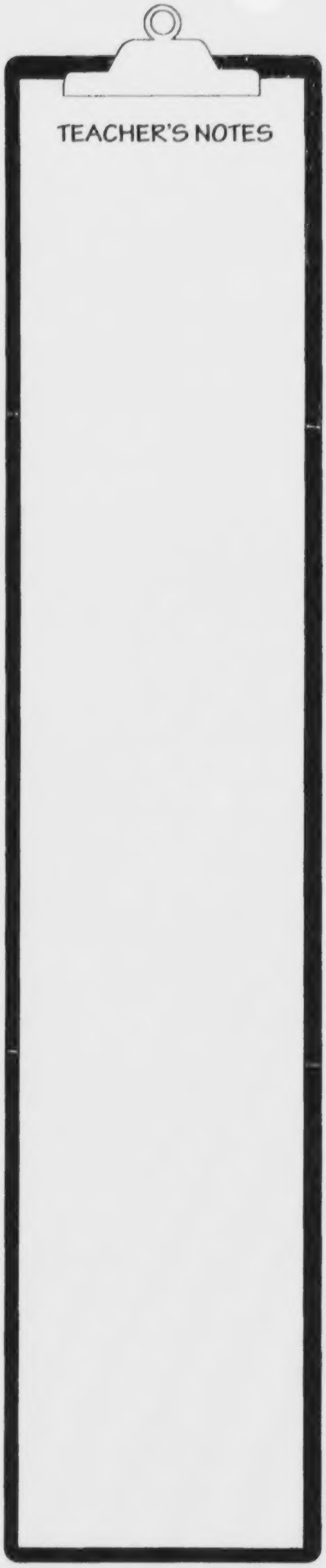
1. Add 6 mL of brine shrimp larvae in 1.0% NaCl culture to 54 mL of 1.0% NaCl and mix them well.
2. Aliquot 10 mL of the diluted culture to each of 6 film cans.
3. Leave 1 film can without an oil addition as the control.
4. To each of the film cans add oil by the drop. Vary the amount of surface covered so that you create 20%, 40%, 60%, 80%, and 100% surface cover.
5. Incubate the brine shrimp in the light for 24 hours.
6. Mix the contents of each container well and count and classify the brine shrimp larvae using the drop counting technique of the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 14. Brine shrimp mortality and survivorship in salt water contaminated with new motor oil.

Percent of surface covered with oil	Number of live brine shrimp	Number of dead brine shrimp
0 (Control)	48	2
20	46	4
40	45	5
60	30	29
80	15	39
100	0	50

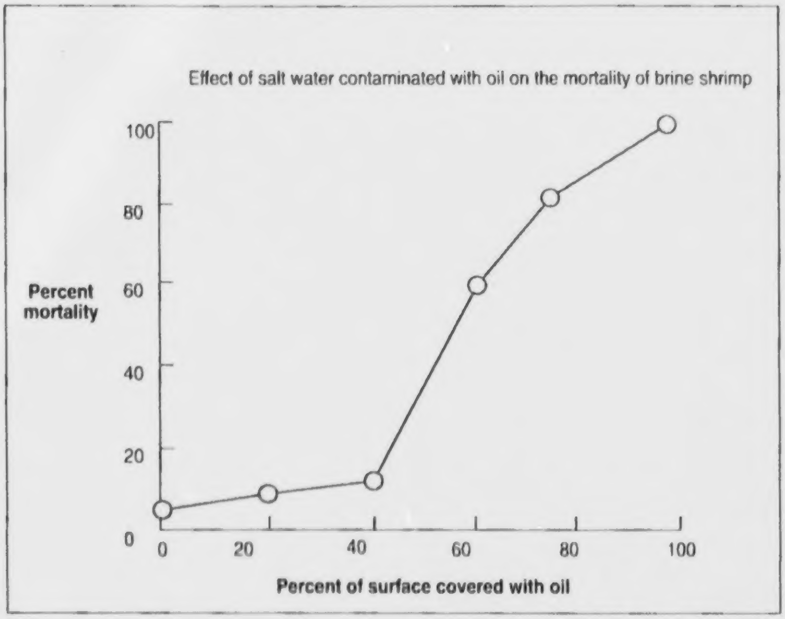


Interpretation

Accept the hypothesis if you calculate a higher percent mortality for higher oil coverage. Reject the hypothesis if you calculate no difference in mortality between treatments or if you calculate a higher percent mortality for greater oil coverage.

Answer to Test Question

Increasing mortality with increasing oil contamination was observed. In some cases, the animals seemed to become coated with the oil so that they could not move well. In other cases, it is suspected that they got oil in their gills so that extracting oxygen was difficult. In the most extreme case of 100% oil coverage, they could have been deprived of oxygen because there was no opportunity for gas exchange between the water and the atmosphere. If the oil were unrefined or used, one would expect that the mortality would have been even higher because toxic materials in the oil would also affect brine shrimp survival.



Graph L. Relationship of percent surface area covered with oil to brine shrimp survival.

TEST QUESTION

Oil pollution of water is a serious problem, whether from maritime accidents or the casual improper disposal of used motor oil. If your brine shrimp mortality increased with increasing oil pollution, what might the cause have been? Would you expect different results if the oil had been unrefined or used?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have students who are visually impaired obtain data for calculations from sighted students.

VARIATION 10

The Effect of Acid Concentrations on Brine Shrimp Survival

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.

SYNOPSIS

Students will examine the effects of increased acid concentration on brine shrimp survival.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 5 mL 1.0% HCl prepared with saline solution
- 100 mL 1.0% NaCl solution

HYPOTHESIS GENERATION

Question

What effect will an increase in acid concentration have on brine shrimp survival?

Sample Hypothesis

An increase in the acid concentration of the stock water will cause a decrease in the number of living brine shrimp in the sample after a 24-hour period.

Rationale

Every organism has an optimum pH for survival. Even a slight decrease in pH may have an adverse effect on an organism's survival.

Sample Experimental Procedure

1. Label your dilution treatment containers to represent the desired final concentrations of acid; include one container with 1.0% sodium chloride, but no acid.
2. Prepare your dilutions as follows (See Figure 10):
   A. Dispense 9 mL of 1.0% sodium chloride into each of your labeled containers.

   B. Add 1 mL of 1.0% acid to the dilution container labeled 0.1%. Mix thoroughly.

   C. Using the same pipette, transfer 1 mL of the 0.1% solution to the dilution container labeled 0.01%.

   D. Use the same pipette to repeat the process of Steps A to C to create your additional dilutions. From 0.01% create 0.001%, and so on.

   E. Remove and discard 1 mL from the weakest solution.

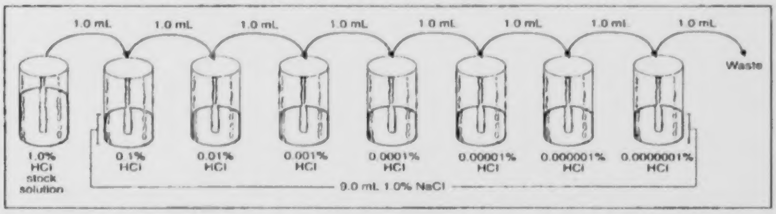


Figure 10. Diagram of dilution solutions.

3. Dispense 1.0 mL of brine shrimp into each dilution.
4. Incubate the containers in light at room temperature for 24 hours.
5. Count the living brine shrimp using the procedures in the Core Experiment listed in Step 7.
6. Determine the survivorship of brine shrimp in each acid concentration. Calculate the percent mortality by dividing the number of dead brine shrimp by the total number of brine shrimp counted. If brine shrimp have died in the control (1.0% NaCl without acid), subtract the percent mortality of the control from the percent mortality of each treatment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

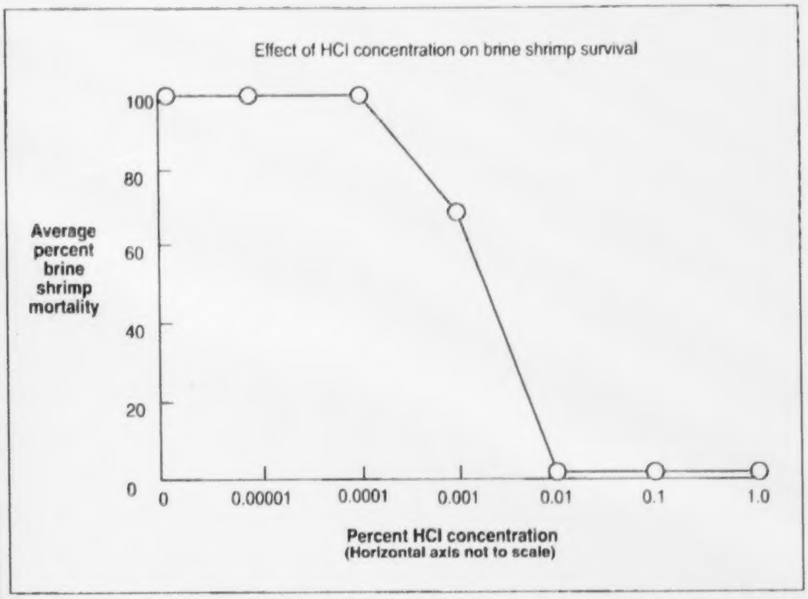
Table 15. Mortality of brine shrimp subjected to various concentrations of hydrochloric acid.

Percent HCl	Number of live brine shrimp	Number of dead brine shrimp	Percent survival = number live/total
1.0	0	50	0
0.1	0	50	0
0.01	0	50	0
0.001	38	12	76
0.0001	50	0	100
0.00001	50	0	100
0.0	50	0	100

TEACHER'S NOTES

Interpretation

The hypothesis is supported by these data. The number of living brine shrimp decreased rapidly as the concentration of the acid increased from 0.0001% to 0.01%. Brine shrimp appear to be sensitive to changes in acidity.



Graph M. Survivorship in solutions of various acid concentrations.

Answer to Test Question

An increase in the concentration of acid causes an increase in the mortality of the brine shrimp. In many aquatic organisms the gills can be injured and respiration may be reduced or stopped.

TEST QUESTION

Discuss the effect of increased acid concentration on brine shrimp.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 9.

Tolerance Testing With A Hairless "Guinea Pig"

Directions for Students

INTRODUCTION

Have you ever had a pet guinea pig? Did you know that they became common in the United States as experimental subjects in biology laboratories before they were popular as pets? Even earlier, they were dinner fare and farmed for that purpose in South America. Maybe you had a pet called a sea monkey, or more likely, you had many of them. Today sea monkeys, also called brine shrimp (*Artemia franciscana*), as shown in Figure 1, have replaced guinea pigs and mice in many labs for toxicity testing of materials. So, brine shrimp are pets and experimental subjects, but are they farmed for table fare also? No, you will not encounter these small creatures on your dinner table, and they are not farmed. You might, however, use them to feed your tropical fish. They are aggressively and competitively harvested for sale to aquarists.

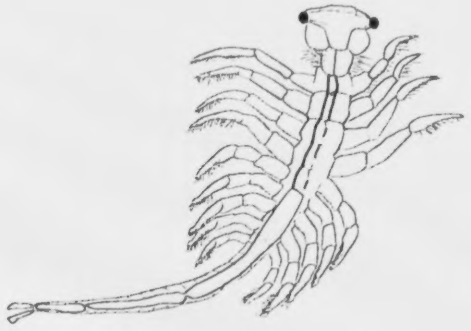


Figure 1. Brine shrimp.

Brine shrimp are found in salt ponds and lakes throughout the world. At the peak of harvest, small planes fly over Utah's Great Salt Lake—a very salty and slightly basic lake—spotting brine shrimp eggs for harvest teams. How can the tiny eggs be harvested with little contamination from other organisms? How many organisms do you suppose can tolerate the high salt concentration of the Great Salt Lake? Is the salinity of a salt pond constant? Are there other changes in an aquatic environment that would affect the ability of different species to survive?

The tiny eggs are really immature, dormant shrimp and will keep for months on the shelf. When put in salt water, they hatch in only 2 or 3 days. Brine shrimp larvae will survive another day or two without food. During this time, they are very sensitive to some materials, such as mining and manufacturing wastes that might pollute water. They also are sensitive to materials that might not be aquatic pollutants, but could be used as drugs to prevent tumor formation in humans. They are used in preliminary assays to screen for anti-cancer potential. These hairless creatures, brine shrimp, are truly modern guinea pigs.

OBJECTIVES

At the end of this lab, you should be able to:

- Define and determine the tolerance limit of *Artemia* larvae to environmental variables, such as salinity.
- Describe how an environmental variable may be a limiting factor in an ecosystem and act as a selective pressure in the survival of a species.
- Develop the skill of using a serial dilution to prepare various concentrations of NaCl solutions.

SAFETY NOTES

Always wear goggles and lab aprons when working with chemicals.

Label all containers so solutions do not get confused.

Always **add acid to water** when preparing acid solutions.

Use caution when mixing the suggested pollutants during the introduction of the Core Experiment. If other "pollutants" are used in addition to those suggested, be aware of any potential for the chemicals to react with one another.

Never mix bleach and ammonia or substances containing either substance; toxic fumes will result.

Use only substances that can be poured safely down the sink.

MATERIALS NEEDED

For each group of two students, you will need the following:

1 10-mL disposable syringe or 10-mL graduated pipette with bulb
1 permanent marker
10 translucent film cans, 50-mL beakers, test tubes, or 1 divided box with 10 chambers
1 teasing needle
1 1-mL graduated plastic pipette
1 stereo microscope
10 mL 8.0% NaCl
40 mL distilled H_2O
1 dropping pipette
1 petri dish with counting grid

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of brine shrimp and the limiting factors in their ecosystem. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. Why is controlling the variables important?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

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QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. How do these data relate to the hypothesis?
2. What caused errors in your experiment?
3. How would you modify your experiment to reduce any errors?
4. What does your graph tell you about survival or hatching of brine shrimp?
5. What other important information did you learn from your experiment?
6. How does this laboratory relate to current class study?
7. What other questions come from your results?
8. To what other biology or science topics is this laboratory related?

DESIGN OF VARIATIONS OF CORE EXPERIMENT

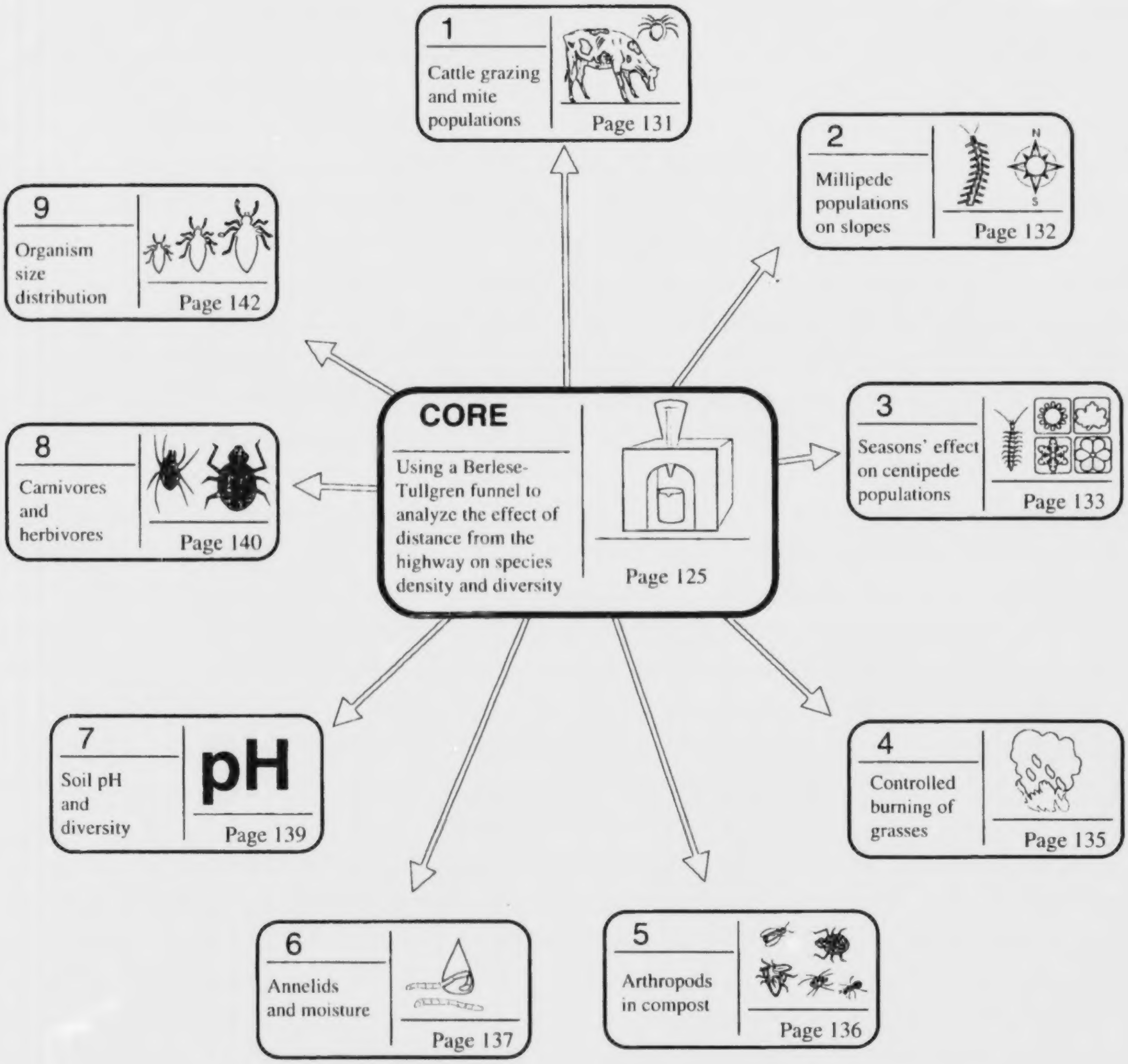
After you have discussed the results of the Core Experiment with your classmates, write down questions that occurred as you tested the various salt concentrations on brine shrimp tolerance. Design an experiment that is quantifiable and write your procedure in a numbered list of steps. Questions other students have studied include the following:

- How will increasing the pH of the water environment, i.e., making it more basic, affect brine shrimp?
- What is the effect of temperature on brine shrimp survival?
- Will *Artemia* show a preference for shallow depths?
- Will increasing phosphate concentration affect the survival rate of brine shrimp?
- What effect will the increase in nitrate concentrations have on brine shrimp?
- What effect do other metal ions have on brine shrimp larvae survival?
- What effect will the substitution of a halogen ion have on the survival rate of brine shrimp?
- What effect will combining a harmful cation with a "safe" anion have on brine shrimp survival?
- What effect will increasing the concentration of oil have on brine shrimp survival?
- What effect will an increase in acid concentration have on brine shrimp survival?

NOTES

A Community Underfoot

At-A-Glance Map of the Core Experiment and Its Variations


CORE
Using a Berlese-Tullgren funnel to analyze the effect of distance from the highway on species density and diversity
Page 125
1
Cattle grazing and mite populations
Page 131
2
Millipede populations on slopes
N
S
Page 132
3
Seasons' effect on centipede populations
Page 133
4
Controlled burning of grasses
Page 135
5
Arthropods in compost
Page 136
6
Annelids and moisture
Page 137
7
Soil pH and diversity
pH
Page 139
8
Carnivores and herbivores
Page 140
9
Organism size distribution
Page 142

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NOTES

A Community Underfoot: Density and Diversity of Invertebrates in Soil or Ground Cover

SYNOPSIS FOR CORE EXPERIMENT

Students will construct Berlese-Tullgren funnels from plastic bottles to extract the invertebrate fauna from soil or ground cover samples. They will determine the effects of specific environmental variables on the diversity of fauna taken at regular intervals away from a major roadway.

APPROPRIATE BIOLOGY LEVEL

Introductory or advanced

TEACHER PARTNERS

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SCIENTIST PARTNER

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Department of Entomology
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Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 131. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Construct a Berlese-Tullgren funnel to sample invertebrate fauna in soil or ground cover samples.
- Estimate the population density and diversity for different environmental conditions.
- Describe the effects of specific environmental variables on a target population or community structure.

MATERIALS NEEDED

For the teacher-led demonstration, you will need the following for a class of 24:

- 1 2-L plastic soda bottle
- 1 pair scissors
- 1 10-cm^2 square of 1/4 or 1/8-inch mesh hardware cloth or plastic needlepoint backing
- 1 pair tin snips
- 1 pair pliers
- 1 10 x 10 x 5-cm soil-surface sample
- 1 trowel or pancake turner
- 1 pair of gloves
- 1 3.8-L (1-gallon) sealable, plastic bag
- 50.0 mL ethanol
- 1 500-mL glass jar with 7.5-cm diameter opening and tight lid
- 1 piece of newspaper
- 1 9-watt colorless light bulb and a socket in a string of holiday lights or a 25-watt shielded light
- 1 10-cm^2 square of aluminum foil or dark paper

You will need the following for each group of two students in a class of 24:

- 3 2-L plastic soda bottles
- 1 pair scissors
- 3 10 x 10 x 5-cm soil-surface samples

(continued on p. 118)

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Introduce the Berlese-Tullgren funnel and key. Practice using the dichotomous key to identify the invertebrate organisms from the demonstration extraction.

Day 2 (45 minutes)

- Analyze data from demonstration sample. Use class totals to estimate population density and diversity. Discuss environmental variables that might influence the density and diversity of these populations.

Day 3 (30 minutes)

- Develop the question, hypothesis, and rationale. Assemble materials for the funnels.

Day 4 (30 minutes)

- Collect sample and place in funnel.

Days 5 to 7

- Allow samples to dry.

Days 8 & 9 (45 minutes)

- Collect and analyze data from the students' extractions.

PREPARATION TIME REQUIRED

30 minutes

- Assemble the demonstration Berlese-Tullgren funnel.

60 minutes

- Collect soil or ground cover sample.

60 minutes

- Cut the screen squares with tin snips and remove the bottoms from bottles.

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TEACHER'S NOTES

(continued from p. 117)

- 1 pair of gloves
- 3 1-L (1-quart) sealable, plastic bags
- 3 10-cm^2 square of 1/4 or 1/8-inch mesh hardware cloth or plastic needlepoint backing
- 3 500-mL glass jars with 7.5-cm diameter openings with tight lids
- 3 9-watt colorless light bulbs and 3 1-socket sections of a string of holiday lights or 3 25-watt shielded lights
- 1 stereoscope
- 1 eyedropper
- 1 forceps
- 1 9 x 14-cm (8.5 x 11-inch) sheet each of black and white construction paper
- 3 10-cm^2 squares of aluminum foil or dark paper
- 5 sheets of newspaper
- 150.0 mL 70% isopropyl alcohol
- 1 garden trowel

SAFETY PROCEDURES

Students should wear gloves whenever handling soil samples and wash their hands at the beginning and end of the lab.

Alcohol is flammable and should not be used near an open flame.

Students should not drink the alcohol or inhale its fumes.

The classroom should have adequate ventilation.

Vapor levels can be reduced by using a collecting jar that fits snugly with the funnel and by keeping collecting jars and petri dishes covered.

If you use a 25-watt light bulb as a heat source over the Berlese-Tullgren funnel, keep the bulb at least 15 cm away from the sample to prevent the ignition of any dry leaves in the sample.

When students collect their soil-surface samples, remind them to disturb as little of the area as possible.

DIRECTIONS FOR SETTING UP THE LAB

Berlese-Tullgren Funnel Assembly

- Cut the bottom from the plastic bottle at the edge of the colored bottom or at the ridge where the sides of the bottle straighten. When the bottle is inverted with the mouth facing downward, it becomes a funnel. See Figure 1. Cut 1-cm slits on opposite sides of the bottle's cut as shown in Figure 1.

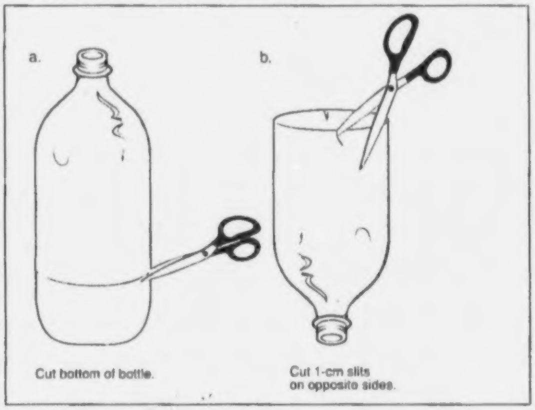
a.
b.
Cut bottom of bottle.
Cut 1-cm slits on opposite sides.

Figure 1. Extraction funnel assembly.

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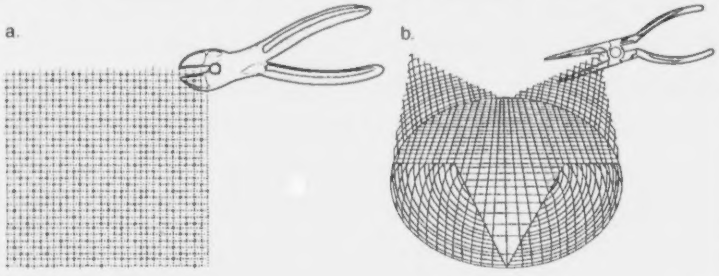
- Use tin snips to cut a 10-cm^2 square from 1/4 or 1/8-inch hardware cloth. See Figure 2a. (Plastic needlepoint backing may be substituted for the hardware cloth. Cut with scissors.)
- Use pliers to bend the edges of the wire into a circle just slightly larger than the funnel's inside diameter. See Figure 2b.

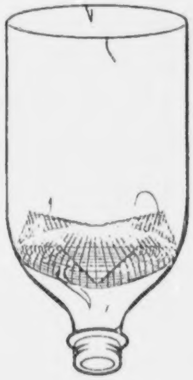


Cut 10-cm square with tin snips.

Use pliers to bend the edges of the wire into a circle.

Figure 2. Cutting the hardware cloth.

- Make a shelf inside the inverted bottle by pushing the hardware cloth into the funnel until it rests on the sides of the bottle where the bottle begins to narrow as shown in Figure 3.



Press screen to where the funnel narrows.

Figure 3. Placement of the hardware cloth.

Sample Collection

Collect a soil-surface sample of moist leaf mold, compost, or rotting wood. Choose a site that is 5 to 6 cm thick. Use a trowel or pancake turner to scrape everything in a 0.5 meter square area above the soil surface into a plastic bag.

Sample Extraction

- Pour about 50.0 mL of alcohol into a 500-mL jar. The alcohol should be about 1.5 cm deep.
- Working over a piece of newspaper, place the soil-surface sample into the funnel. As the fine sample particles fall through the screen onto the newspaper, return

TEACHER'S NOTES

TEACHER'S NOTES

them to the funnel. After all the loose material has stopped falling, set the funnel on top of the alcohol jar.

- Center the light over the funnel and anchor the cord in the slits that were cut. Set the bottom of the funnel over the light. This will provide a shield to keep insects attracted by the light from contaminating your sample. See Figure 4.

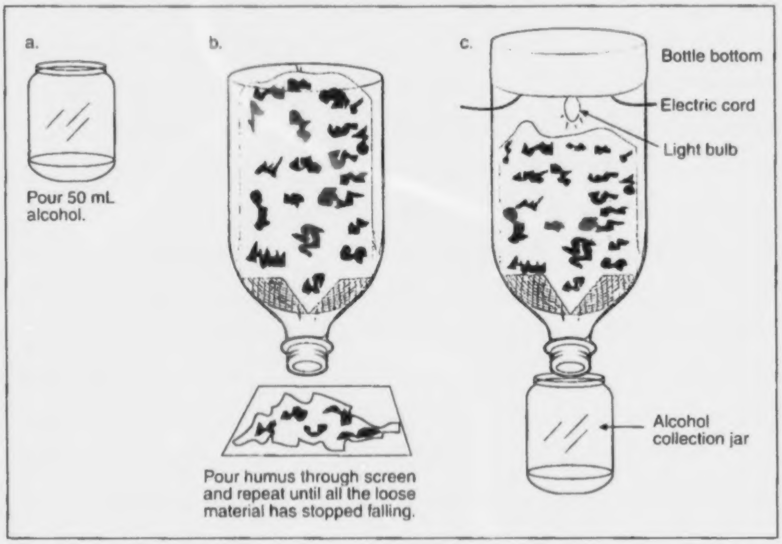

a.
Pour 50 mL alcohol.
b.
Pour humus through screen and repeat until all the loose material has stopped falling.
c.
Bottle bottom
Electric cord
Light bulb
Alcohol collection jar

Figure 4. Extraction funnel setup.

- Wrap foil or dark paper around the jar to make a dark refuge for the invertebrates that are negatively phototropic. See Figure 5a.
- As the sample dries on the top, the animals will move downward through the screen into the alcohol. Check the alcohol level daily and add more if it evaporates. After 4 or 5 days, carefully remove the alcohol collection jar and screw on its lid. See Figure 5.

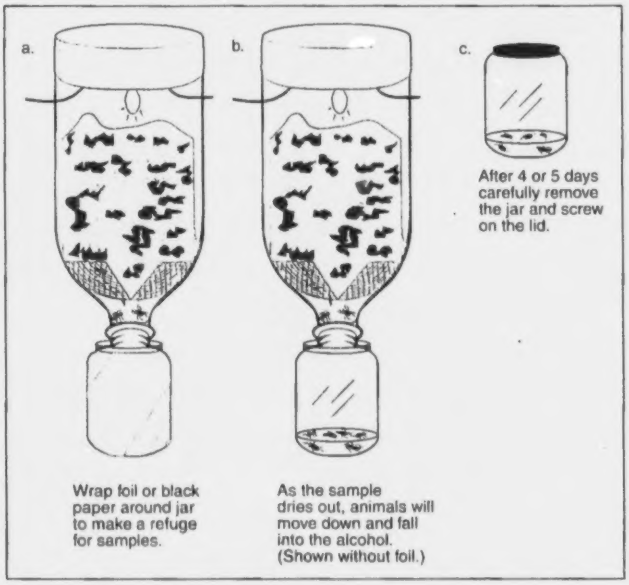

a.
Wrap foil or black paper around jar to make a refuge for samples.
b.
As the sample dries out, animals will move down and fall into the alcohol. (Shown without foil.)
c.
After 4 or 5 days carefully remove the jar and screw on the lid.

Figure 5. An extraction funnel with a sample.

TEACHER BACKGROUND

Content Information

Beneath our feet lives a world of organisms most people have never seen. Every major animal phylum, except the Cnidaria and Echinodermata, has members in the below-ground ecosystem. Numerous Protista and Monera are also abundant there. From a single 30-cm^2 sample of the 2.5 to 5.0 cm of humus that covers a typical temperate deciduous forest or grassland, representatives of 15 to 20 major taxonomic groups may be collected. The population density of some species may exceed one million individuals per acre.

Among these soil organisms are representatives of every trophic level including producers, primary and secondary consumers, and decomposers. Population growth is limited by environmental conditions and the amount of resources available. Invertebrate animals may be herbivores, predators, parasites, or saprophytes. They may consume roots, seeds, fungi, dead wood, decaying leaves, or each other. The soil community is a microcosm of the complex ecological interactions among living organisms. The density of the population is not fixed, but varies with climate, season, and environmental factors, such as light, water, space, and nutrients.

Even though the soil surface is densely populated, many of its inhabitants can be found only by extraction and observed only under magnification. At the end of the 19th century, the Italian entomologist, Antonio Berlese, developed the first funnel-type extraction system for separating small arthropods from the soil. Hot water circulated between double brass walls of his funnel. A sample of soil or leaf litter was placed in the cavity of the funnel that was then suspended over a jar of alcohol. Heat, conducted from the water through the metal walls of the funnel, caused the moisture in the sample to evaporate. Arthropods and other small invertebrates moved downward through the funnel to escape desiccation and eventually fell into the alcohol where they were killed quickly and preserved.

Fillippo Silvestri, a student of Antonio Berlese, used this extraction system in 1907 while he was studying the soil fauna of the woodlands near Syracuse, New York. Some of his samples contained an undescribed arthropod species that proved to be the first identified representative of an entirely new taxonomic order, the Protura. These tiny insects are found only in moist leaf litter and are considered to be the most primitive group of Hexapods. So far, 20 species have been discovered on the North American continent.

In 1918, Tullgren modified Berlese's original extraction method by replacing the water jacket with an electric light bulb. All commonly used extraction funnels depend on the differential light and moisture created by placing a water-based collection vat beneath a funnel and an electric light and heat source over the funnel. You will find literature references to both Berlese funnels and Tullgren funnels. We honor both innovators by calling the extraction apparatus a Berlese-Tullgren funnel.

Collembola (springtails) are probably one of the most abundant animals collected in Berlese samples. Approximately 1,500 species of the order Collembola have been identified (Lovett, 1993) and their population density often exceeds 1 million per acre. In some grasslands, they have been found in densities as high as 300 million per acre (Wallwork, 1970). This primitive, wingless insect has a unique "springtail" near the end of its abdomen that it can snap against the substrate, propelling itself through the air. Some Collembola can jump up to 20 centimeters, a distance 50 to 100 times their own body length. Unlike most other arthropods, springtails appear to have evolved in cool climates and their relative abundance in the soil tends to increase as the mean annual temperature decreases. Today, these animals are even found in the Arctic and Antarctic (Lovett, 1993).

TEACHER'S NOTES

Many of the animals are so tiny that you easily could overlook them even if they were separated from their surrounding litter or soil. Ninety-five percent of them are less than 5 millimeters long and nearly 37% of them are even smaller than 1 millimeter (Birch & Clark, 1953).

Extractions of humus collected from a temperate deciduous forest are likely to contain individuals from some or all of the following taxonomic groups:

- Nematoda (roundworms)
- Annelida (segmented worms)
- Mollusca (snails)
- Arthropoda (spiders, ticks, mites, daddy longlegs, pill bugs, sow bugs, woodlice, centipedes, silverfish, fleas)
- See the *Kwik-Key to Soil-Dwelling Invertebrates* (Figure 7a & b) for detailed information.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts:

Correct Concept	Misconception
• The soil surface is densely populated with animals. Soil animals are hard to see.	• The soil surface contains only nonliving material. • Very few animals live on the soil surface.
• Many different types of animals live on the soil surface.	• All soil-surface animals are the same. Only worms live in the soil.
• The organisms living in soil form a microcosm of the larger ecosystem and are also part of that ecosystem.	• The soil-surface environment is not an ecosystem or part of the larger ecosystem. • All soil-surface organisms fill the same trophic level and make no contribution to the larger ecosystem.

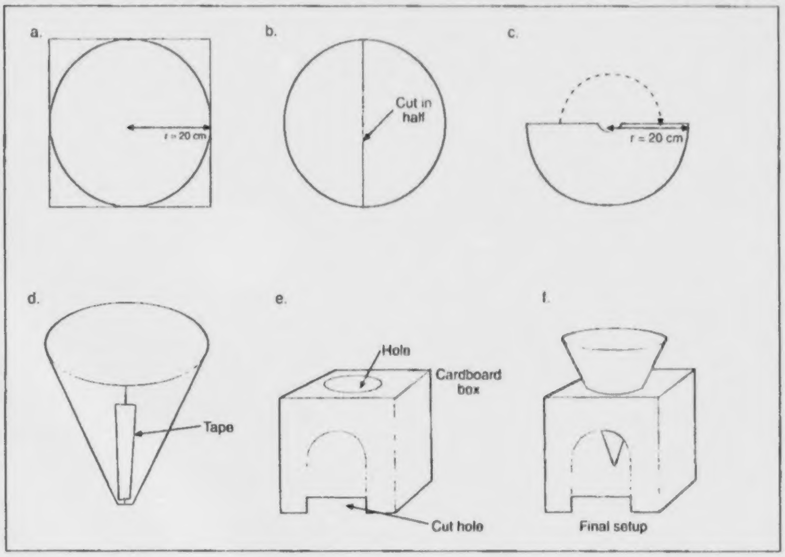


Figure 6. Extraction funnel and stand made from acetate or sheet vinyl and a cardboard box.

TEACHING TIPS

- Selection of the sample sites is critical to the success of this laboratory activity. More organisms will be found in samples from sites that are somewhat sheltered and not subject to periodic flooding or desiccation. High population density and high species diversity usually can be found in the following materials:
  - leaf mold associated with neutral soils in deciduous forests
  - leguminous ground covers such as clover, vetch, alfalfa
  - compost piles with a mixture of vegetable matter
  - mulch from vegetable gardens or organic farms.
- Prior experience with dichotomous keys and/or cladistics is helpful.
- Samples that are too damp or dense do not work well. In some cases, a larger sample (25 cm^2) may be needed to see a good cross-section of animals.
- All Berlese-Tullgren funnels should be set up in a location where they will not be disturbed. Excessive agitation will cause the dirt to loosen and fall into the alcohol. This will cloud the final sample and make identification of the organisms more difficult.
- May is the time suggested by field testers for this lab. The soil is warmer and invertebrates are more active.
- Berlese-Tullgren funnels that can accommodate larger soil-surface samples can be constructed from inexpensive materials such as sheets of clear acetate or scraps of vinyl flooring. Cut the material into a half-circle, as illustrated in Figure 6. Then, join the radii and use cellophane shipping tape or vinyl duct tape along the seam to form a funnel. Make the radius of a half-circle equal to the desired diameter of the finished funnel. A 40-cm diameter funnel will be large enough to hold a 30 x 30-cm sample. Make a stand for the funnel by cutting a circle in the top of a cardboard box.
- A Berlese-Tullgren funnel for a small humus sample can be made using the above procedure with 15-cm diameter filter paper. Punch a small hole at the point of the funnel. A cone-shaped coffee filter may be substituted for the filter paper.

(continued on p. 125)

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Kwik-Key to Soil-Dwelling Invertebrates

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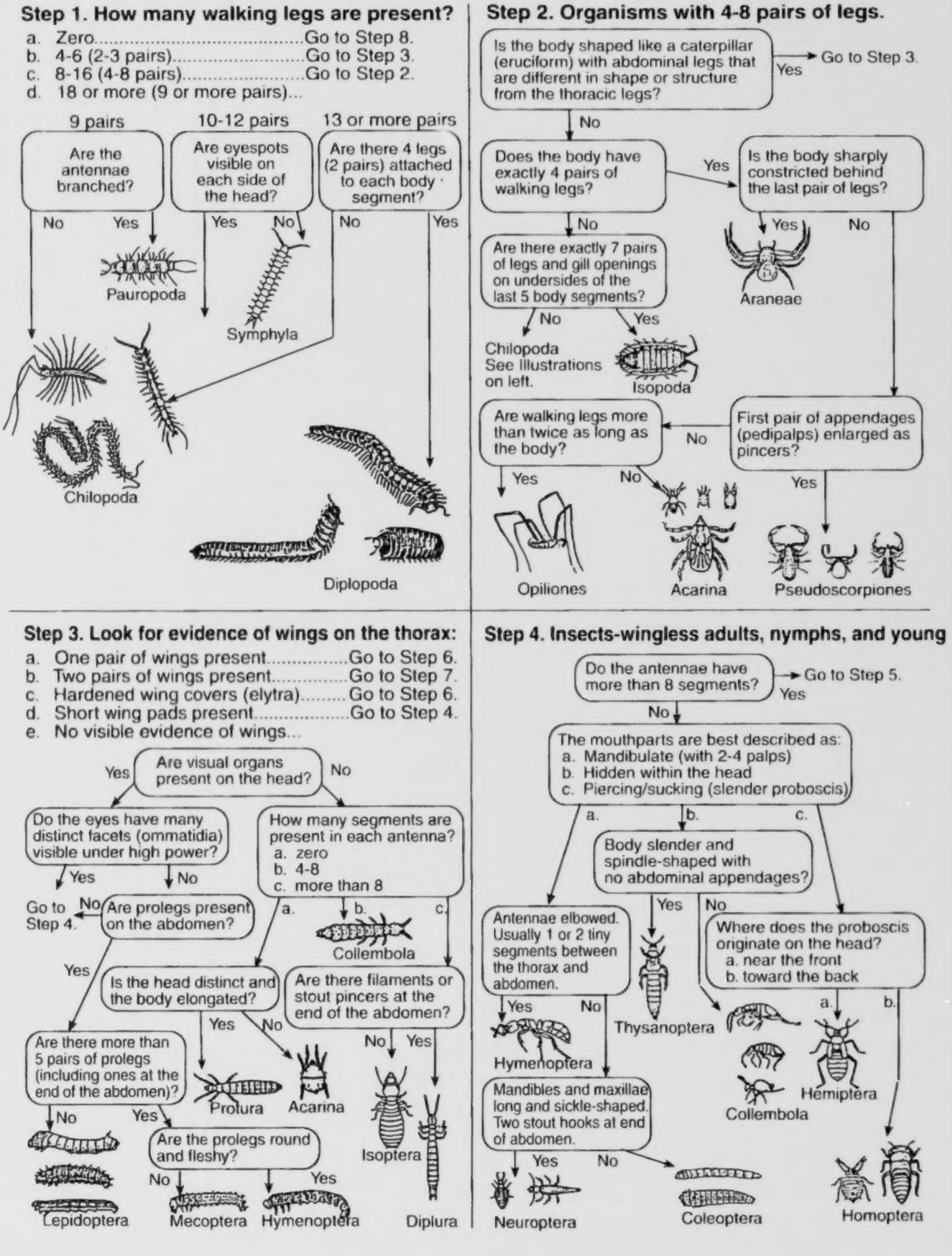
Step 1. How many walking legs are present?
a. Zero....................Go to Step 8.
b. 4-6 (2-3 pairs)....................Go to Step 3.
c. 8-16 (4-8 pairs)....................Go to Step 2.
d. 18 or more (9 or more pairs)...
9 pairs
10-12 pairs
13 or more pairs
Are the antennae branched?
Are eyespots visible on each side of the head?
Are there 4 legs (2 pairs) attached to each body segment?
No
Yes
Yes
No
No
Yes
Pauropoda
Symphyla
Chilopoda
Diplopoda
Step 2. Organisms with 4-8 pairs of legs.
Is the body shaped like a caterpillar (eruciform) with abdominal legs that are different in shape or structure from the thoracic legs?
Yes
Go to Step 3.
No
Does the body have exactly 4 pairs of walking legs?
Yes
Is the body sharply constricted behind the last pair of legs?
Yes
No
Araneae
No
Are there exactly 7 pairs of legs and gill openings on undersides of the last 5 body segments?
No
Yes
Chilopoda See Illustrations on left.
Isopoda
First pair of appendages (pedipalps) enlarged as pincers?
No
Are walking legs more than twice as long as the body?
Yes
No
Yes
Opiliones
Acarina
Pseudoscorpiones
Step 3. Look for evidence of wings on the thorax:
a. One pair of wings present....................Go to Step 6.
b. Two pairs of wings present....................Go to Step 7.
c. Hardened wing covers (elytra)..........Go to Step 6.
d. Short wing pads present....................Go to Step 4.
e. No visible evidence of wings...
Are visual organs present on the head?
Yes
No
Do the eyes have many distinct facets (ommatidia) visible under high power?
Yes
No
Go to Step 4.
No
Are prolegs present on the abdomen?
Yes
How many segments are present in each antenna?
a. zero
b. 4-8
c. more than 8
a.
b.
c.
Collembola
Is the head distinct and the body elongated?
Yes
No
Are there filaments or stout pincers at the end of the abdomen?
No
Yes
Are there more than 5 pairs of prolegs (including ones at the end of the abdomen)?
No
Yes
Are the prolegs round and fleshy?
No
Yes
Protura
Acarina
Isoptera
Diplura
Lepidoptera
Mecoptera
Hymenoptera
Step 4. Insects-wingless adults, nymphs, and young
Do the antennae have more than 8 segments?
Yes
Go to Step 5.
No
The mouthparts are best described as:
a. Mandibulate (with 2-4 palps)
b. Hidden within the head
c. Piercing/sucking (slender proboscis)
a.
b.
c.
Body slender and spindle-shaped with no abdominal appendages?
Yes
No
Thysanoptera
Antennae elbowed. Usually 1 or 2 tiny segments between the thorax and abdomen.
Yes
No
Hymenoptera
Where does the proboscis originate on the head?
a. near the front
b. toward the back
a.
b.
Collembola
Hemiptera
Mandibles and maxillae long and sickle-shaped. Two stout hooks at end of abdomen.
Yes
No
Neuroptera
Coleoptera
Homoptera

Figure 7a. *Kwik-Key to Soil-Dwelling Invertebrates* (Steps 1 through 4).

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Kwik-Key to Soil-Dwelling Invertebrates

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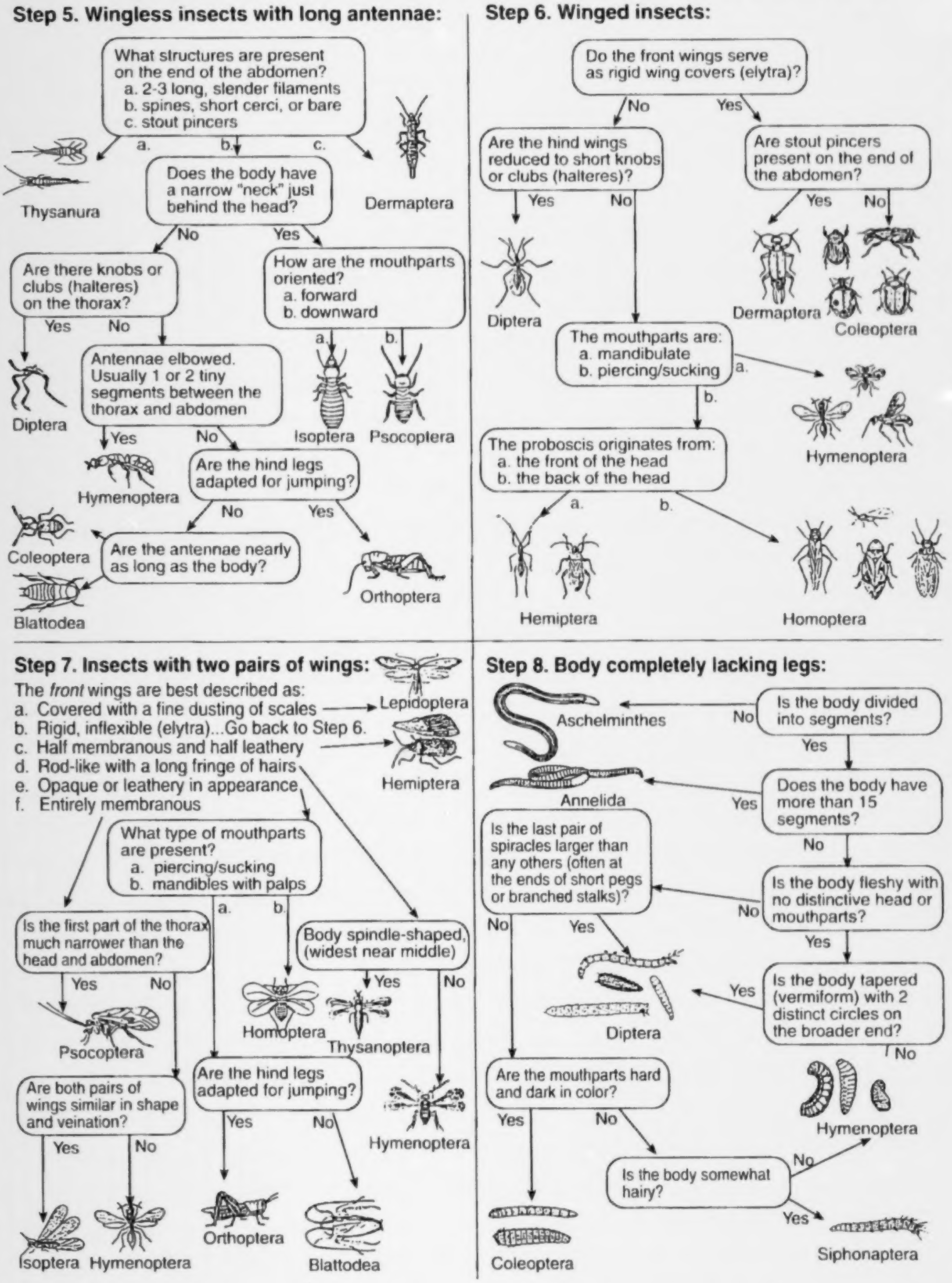
Step 5. Wingless insects with long antennae:
What structures are present on the end of the abdomen?
a. 2-3 long, slender filaments
b. spines, short cerci, or bare
c. stout pincers
a.
b.
c.
Thysanura
Dermaptera
Does the body have a narrow "neck" just behind the head?
No
Yes
Are there knobs or clubs (halteres) on the thorax?
Yes
No
Diptera
How are the mouthparts oriented?
a. forward
b. downward
a.
b.
Isoptera
Psocoptera
Antennae elbowed. Usually 1 or 2 tiny segments between the thorax and abdomen
Yes
No
Hymenoptera
Are the hind legs adapted for jumping?
No
Yes
Orthoptera
Are the antennae nearly as long as the body?
Coleoptera
Blattodea
Step 6. Winged insects:
Do the front wings serve as rigid wing covers (elytra)?
No
Yes
Are the hind wings reduced to short knobs or clubs (halteres)?
Yes
No
Diptera
Are stout pincers present on the end of the abdomen?
Yes
No
Dermaptera
Coleoptera
The mouthparts are:
a. mandibulate
b. piercing/sucking
a.
b.
Hymenoptera
The proboscis originates from:
a. the front of the head
b. the back of the head
a.
b.
Hemiptera
Homoptera
Step 7. Insects with two pairs of wings:
The *front* wings are best described as:
a. Covered with a fine dusting of scales
b. Rigid, inflexible (elytra)...Go back to Step 6.
c. Half membranous and half leathery
d. Rod-like with a long fringe of hairs
e. Opaque or leathery in appearance
f. Entirely membranous
Lepidoptera
Hemiptera
What type of mouthparts are present?
a. piercing/sucking
b. mandibles with palps
a.
b.
Is the first part of the thorax much narrower than the head and abdomen?
Yes
No
Psocoptera
Homoptera
Body spindle-shaped, (widest near middle)
Yes
No
Thysanoptera
Hymenoptera
Are both pairs of wings similar in shape and veination?
Yes
No
Isoptera
Hymenoptera
Are the hind legs adapted for jumping?
Yes
No
Orthoptera
Blattodea
Step 8. Body completely lacking legs:
Is the body divided into segments?
No
Yes
Aschelminthes
Does the body have more than 15 segments?
Yes
No
Annelida
Is the body fleshy with no distinctive head or mouthparts?
No
Yes
Is the last pair of spiracles larger than any others (often at the ends of short pegs or branched stalks)?
No
Yes
Diptera
Is the body tapered (vermiform) with 2 distinct circles on the broader end?
Yes
No
Hymenoptera
Are the mouthparts hard and dark in color?
Yes
No
Coleoptera
Is the body somewhat hairy?
No
Yes
Siphonaptera

Figure 7b. *Kwik-Key to Soil-Dwelling Invertebrates* (Steps 5 through 8).

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INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Have the Berlese-Tullgren funnel set up in front of the class when students arrive. Ask them to describe the apparatus and explain how you set it up. Challenge students to determine the function of the apparatus.

Discuss the soil-surface sample and describe its collection site. Show them a map of the site if one is available. Allow students to inspect the sample briefly, then ask the following questions:

- What organisms do you think live in the environment of the soil-surface sample?
- What would affect the number or kinds of organisms in a sample?
- What are the organisms eating?
- What evidence do you find that supports your answers?

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

Students should identify a site to collect a sample of soil surface or leaf litter and list possible factors that may affect the sample. Then, they should form a hypothesis about the effects of a variable that was present at that site. If necessary, you may suggest examples from the following lists.

Study sites might include:	**Variables might include:** **Natural**	 **Artificial**
• Agricultural fields • Organic farms • Compost piles • Grasslands or pastures • Orchard ground cover • Hardwood forest leaf litter • Coniferous forest pine straw • Wetlands (bogs or swamps) • Lawns or golf courses	• Time of year • Grazing • Shading • Elevation • Slope face (north vs south) • Degree of slope (flat to steep) • Location on a slope (top vs bottom)	• Physical disturbance • Addition of chemicals • Mulching • Mowing • Nearby disturbance • Introduction of exclusion of organisms

As students develop a hypothesis, they should decide what they will measure to test it. Possible measurable elements include:

- population age structure determined by:
  - measuring the head
  - counting the body segments
- species diversity calculated by using diversity indexes
- population density of a selected species determined by:
  - extrapolating from sampling counts
  - using statistical analysis
- rate of development or change determined by observing population dynamics
- trophic structure determined by:
  - developing a pyramid of numbers or biomass
  - counting the relative numbers of predators and preys

TEACHING TIPS

(continued from p. 122)

- Provide black and white construction paper for students to place under their petri dishes while examining the extracted sample. Light-colored organisms should be more visible with a dark background; darker organisms should be more visible with a white background.
- Scratch a grid on the bottom of the petri dish to help students keep track of their search progress. A dissecting needle or straight pin are good tools for this. A piece of graph paper attached to the inside of the petri dish will provide a guide for your grid pattern. Five squares to 2.5 cm (1 inch) is fine enough for most searches.
- Gently swirl the petri dish of alcohol containing the sample on the microscope stage. Most of the animals will concentrate near the center of the dish.
- Most animals will sink and can be seen easily on the bottom of the petri dish, but Collembola float and can be found on the surface. Wash down the sides of the collection vial to dislodge any organisms adhering to the sides of the dish.
- Students can estimate population density for each taxon using the factor-label method. For example, 36 mites collected in a sample measuring 15 x 15 cm (225 cm^2) would use the following conversion:

$$\frac{36 \text{ mites}}{225\text{cm}^2} \times \frac{10{,}000 \text{ cm}^2}{1\text{m}^2}$$
$$= 1{,}600 \text{ mites/m}^2$$

- Pool class data and average all values from similar habitats to give a more accurate estimate of population density. Extrapolate this estimate to number/acre or number/hectare by using the appropriate conversion factors:

1 hectare = 2.471 acres
= 10,000 square meters
1 acre = 4047 square meters
= 0.4047 hectare

- An illustrated key for identifying soil-dwelling invertebrates to class or order is used in this lab. The *Kwik-Key to Soil-Dwelling Invertebrates* illustrated in Figures 7a & b includes a synopsis of the life history and ecology for each taxon. This key and 2 x 2 color transparencies of various soil-dwelling organisms may be purchased from Vision Press, PO Box 5554, Raleigh, NC 27650.

TEACHER'S NOTES

Sample Hypotheses

- Leaf litter or a soil-surface sample collected from a suburban area treated with pesticides will have fewer living organisms than a sample from an untreated suburban area.
- Leaf litter or a soil-surface sample collected from the schoolyard will have less diversity of organisms than a sample collected from an isolated wooded area.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 129.

Question

Does a roadway have an effect on the soil communities living nearby?

Hypothesis

Species diversity and density increase with increasing distance from a highway.

Rationale

Motor vehicles will produce a variety of pollutants that will be introduced to adjacent communities. The farther a community is from the road, the less likely it will be exposed to these pollutants.

Procedure

1. Construct 3 Berlese-Tullgren funnels following the demonstration instructions.
2. Collect leaf litter or soil-surface samples at regular intervals from the roadway.
3. Place the sample in the funnels.
4. Allow the samples to dry out for 5 days. Make observations each day of the number of organisms that have fallen into each collection jar.
5. After 5 days, remove the funnels. Count and identify all the organisms that have fallen into each collection jar.
6. Analyze data to measure density and diversity and compare various samples.
7. (*Optional*) Analyze diversity using Simpson's Diversity Index.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 1. The diversity of organisms at three distances from a highway.

Taxonomic group	Number of species present at a distance of:		
	1 Meter	2 Meters	3 Meters
Subphylum Chilicerata	7	6	5
Subphylum Crustaceae	0	0	1
Subphylum Uniramia: **Classes Chilopoda and Diplopoda**	3	6	6
Subphylum Uniramia: **Class Insecta**	5	14	24

To analyze these data calculate the total number of species at each distance from the highway.

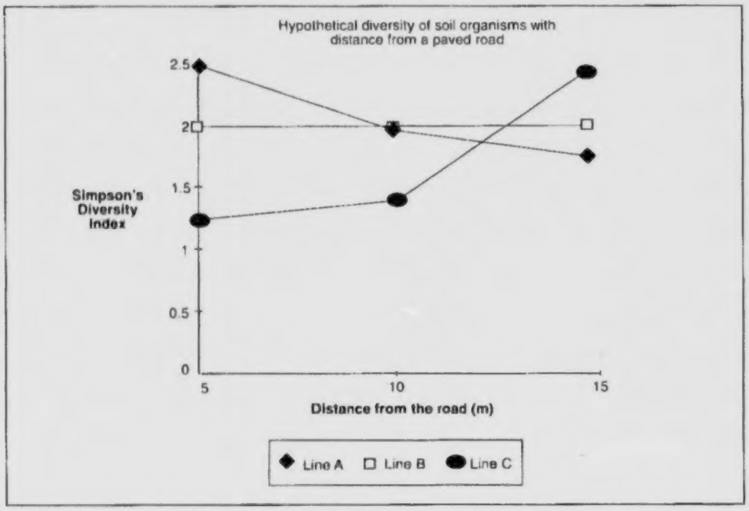
Advanced students can compute an Index of Diversity for comparing the samples. One index they are likely to have appropriate data for is Simpson's Diversity Index. The formula for calculating this index is:

$$N_2 = \frac{N(N-1)}{\sum n(n-1)}$$

where: N_2 = *Simpson's Diversity Index*
N = Total number of individuals of all species
n = Number of individuals of a species

TEST QUESTION

Which of the following lines represents an increasing species diversity with increasing distance from the highway?



Graph A. Hypothetical diversity of soil organisms with distance from a paved road.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think about questions that occurred to them as they conducted their first experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- What did you find out about the density of soil communities near a roadway?
- What did you find out about the diversity of soil communities near a roadway?
- What do you think you would find if you selected a different roadway?
- What are some of the factors that might have affected the density and diversity in soil communities?
- What other factors might affect the density and diversity in soil communities, e.g. soil moisture, pH, mowing, light, seasons, animal grazing, disturbed vs. undisturbed, developed vs. undeveloped?

Interpretation

The hypothesis should be accepted if the number of species collected decreases in samples closer to the highway. The hypothesis should be rejected if the number of species is similar in all the samples or if the number increases closer to the highway.

Answer to Test Question

Line A.

TEACHER'S NOTES

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

- Excuse blind or severely visually impaired students from this experiment due to the complicated nature of identifying microscopic organisms. Organisms are too small to be identified by touch. Their sounds are not audible enough for identification. There is also the chance that the student may be bitten by one of the insects. An alternate experiment would be to do a behavioral study by changing the size and species of animals.

Deaf or Hard-of-Hearing

- Provide for easy communication within the lab team, especially when participants are in the field.

Gifted

- Challenge students to explore more complex habitats and to design multivariate experiments.
- Distinguish between measures of frequency, abundance, constancy, and fidelity.
- Discuss the difference between abundance or population per unit area and relative abundance or percent of the total sample.
- Use binomial confidence intervals to illustrate how reliability of a population estimate increases as the number of samples increases.
- Look for a relationship between frequency and abundance by plotting these statistics on opposing axes. Use classroom data for several taxa from a series of similar Berlese-Tullgren samples.
- Compare several diversity indices and determine which ones are most sensitive to changes in sample size.
- Identify species associations by using correlation statistics on Berlese-Tullgren data collected from different habitats.

Mobility Impaired

- Equip the wheelchair with a sturdy tray to allow the mobility impaired student to work on soil samples supplied by lab team members. "Dirt tires" make many areas accessible for the wheelchair-bound student.
- Encourage mobility impaired students to assist with the construction and use of the Berlese-Tullgren funnel.
- Encourage lab team members when asked to assist physically and mobility impaired students with observations of the invertebrates that are separated from the soil samples and with locating them under the microscope.
- Provide physically impaired students with the *Kwik-Key to Soil-Dwelling Invertebrates* to assist in identifying the small organisms.
- Place organisms under a magnifying glass mounted on a stand.
- Have other students photograph the site for later reference. The photo should include students collecting samples.
- Select a site that will facilitate mobility for students in wheelchairs.
- Use a video microscope camera to display organisms on a large screen.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. Describe how a Berlese-Tullgren funnel works and its use. Provide an illustration in your answer.
2. Write a simple key to separate and identify the following four types of organisms.

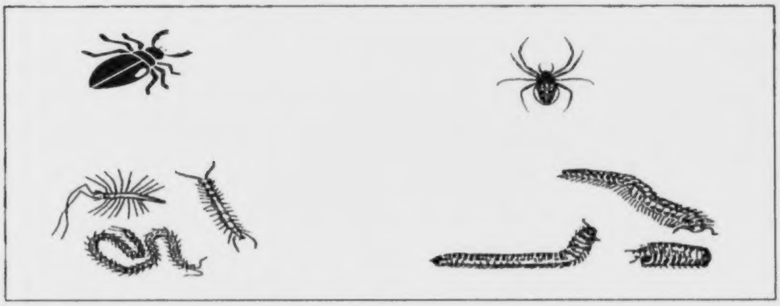


Figure 8. Four common types of arthropods.

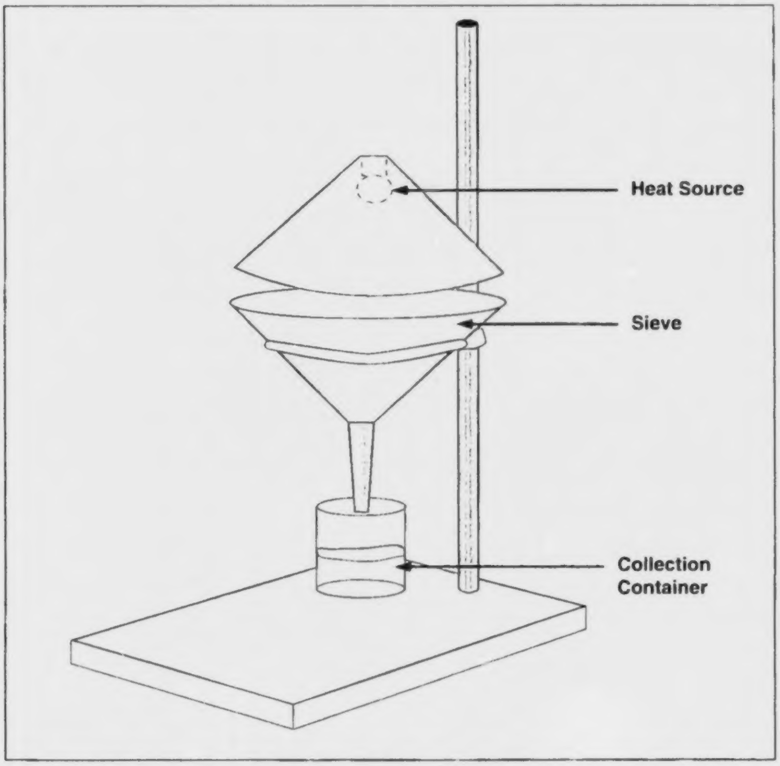


Figure 9. A Berlese-Tullgren funnel has a heat source, a sieve to support the samples, and a container of alcohol to collect specimens.

Answers to Additional Test Questions

1a. A Berlese-Tullgren funnel is used to extract invertebrate animals from a leaf litter or soil-surface sample. It works in the following way:

- Place a sample of soil in the funnel and suspend it over a jar of alcohol.
- Place a light over the sample so that the sample will dry out slowly from the top down.
- The invertebrate animals in the sample will move downward to escape the dryness and fall into the alcohol where they will be preserved.

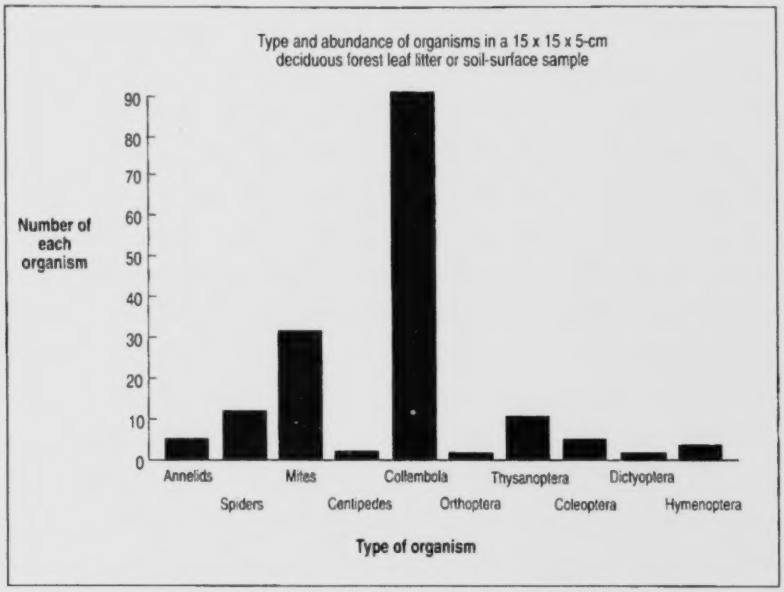
1b. See Figure 9 for a sample setup.

2. Possible student key:

1a. fewer than 10 legs....................2
1b. more than 10 legs....................3
2a. 6 pairs of legs....................insect
2b. 8 pairs of legs...................spider
3a. 1 pair of legs per segment...............................centipede
3b. 2 pairs of legs per segment................................millipede

Answers to Questions and Analysis on Student Page

1. The answer will be a bar graph based on the students' data. The graph will vary depending on these data. The graph title and axes labels should be similar to those of Graph B.
2. Yes.
3. Answers will vary.
4. Number of organisms in the sample the teacher set up will vary because each group analyzes only a part of the sample. In samples collected by students, answers will vary also due to natural variation within a habitat and variation across habitats. For example, within a habitat, dry and wet areas in the same location will provide different results.
5. The more samples you have, the more your data will represent the habitat accurately.
6. No.
7. Location, season, weather conditions, and time of day are some examples of differences.
8. Diverse organisms exist in the soil-surface sample. Sampling is very important in a scientific experiment for accurate results. Lots of different variables will affect the results of these data. Answers will vary based on class results.
9. Answers will vary. One possible answer is the following: Many invertebrates inhabit the soil surface. Some examples are springtails, earthworms, and pill bugs. Some invertebrates are carnivores, some are herbivores.



Graph B. The abundance of diverse soil organisms in a spring sample collected by high school students.

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VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: *Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.*

VARIATION 1

The Effect of Cattle Grazing on the Population Density of Oribatid Mites

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from a pasture where cattle are grazing and from adjacent land where cattle are excluded. They will count the number of oribatid mites in each sample.

HYPOTHESIS GENERATION

Question

How will cattle grazing affect density of mites in a pasture?

Sample Hypothesis

Grazing cattle reduce the density of oribatid mites in a pasture.

Rationale

Students should develop their own rationale for the hypothesis.

Sample Experimental Procedure

Collect soil-surface samples from cattle-grazed and nearby ungrazed pasture. Follow the sample procedure from the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 2. The density of oribatid mites in adjacent grazed and ungrazed pastures.

Replicate	Treatment (grazed)	Control (ungrazed)
1	25	19
2	17	14
3	34	32
4	21	28
5	13	6
Mean	22	19.8

Analyze these data using the mean and the variance for the treatment and the control. Use average values to estimate the population density of the mites.

TEACHER'S NOTES

Interpretation

Accept the hypothesis if the mean number of mites in the control is higher than in the treatment. This suggests that grazing influences the population size of oribatid mites. Reject the hypothesis if the mean number of mites in the control is equal to or lower than in the treatment.

TEST QUESTION

A student hypothesizes that Collembola density will decline after clear cutting a forest. Do the following data support this hypothesis?

Table 3. Density of Collembola before and after clear cutting in a hypothetical location.

	Number of Collembola	
Sample number	Before clear cutting	After clear cutting
1	25	19
2	34	30
3	17	14

Answer to Test Question

The hypothesis is supported. There are fewer Collembola after clear cutting than before clear cutting.

VARIATION 2

The Density of Millipedes on North- and South-Facing Slopes

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from north- and south-facing slopes near the peak of a dome-shaped hill or mountain. They will identify and count all Diplopoda.

HYPOTHESIS GENERATION

Question

Is millipede density affected by exposure to north- or south-facing slopes?

Sample Hypothesis

Millipedes are less dense on north-facing slopes than on south-facing slopes.

Rationale

Students should derive their own rationale for the hypothesis.

Sample Experimental Procedure

Collect soil-surface samples from the north- and south-faces of adjacent hillsides. Follow the sample procedure from the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 4. The hypothetical density of millipedes on north- and south-facing slopes.

Millipede	North	South
Brown species		
Replicate 1	3	7
Replicate 2	5	8
Replicate 3	2	10
Ivory species		
Replicate 1	5	5
Replicate 2	3	7
Replicate 3	6	5
Banded species		
Replicate 1	8	2
Replicate 2	10	3
Replicate 3	9	0

Analyze these data by calculating the mean and the range for the numbers of individuals belonging to each species in each location.

TEST QUESTION

A student hypothesizes that fewer spiders will be found on a north-facing slope than on a south-facing slope. How could the student design an experiment to test this hypothesis?

VARIATION 3

The Seasonal Changes in the Age Structure of a Centipede Population

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will collect and process Berlese-Tullgren soil-surface samples from the same site at monthly or seasonal intervals. They will count the number of body segments and/or measure head capsule width for all members of one centipede species.

HYPOTHESIS GENERATION

Question

Is the age of a generation of centipedes similar?

Sample Hypothesis

If there is only one generation of centipedes per year and centipedes live less than one year, all members of the population should be similar in age.

Interpretation

The hypothesis should be accepted if a higher millipede density is found in samples collected on south-facing slopes. This may suggest that the warmer micro-climate favors millipede growth and development. Reject the hypothesis if similar populations were found in both areas or if higher densities were found in north-facing samples. This may suggest that millipedes are not sensitive to the temperature of the micro-climate or that they prefer conditions that are cooler and more humid.

Answer to Test Question

Construct an experimental setup with samples taken from north-facing and south-facing slopes. Count the number of spiders in each sample and compare these data.

TEACHING TIPS

- Do not suggest a Chi-Square Goodness of Fit analysis unless there are at least 5 individuals in each count.
- External calipers will be useful for measuring the width of the head capsule.

Rationale

Students should derive their own rationale for the hypothesis.

Sample Experimental Procedure

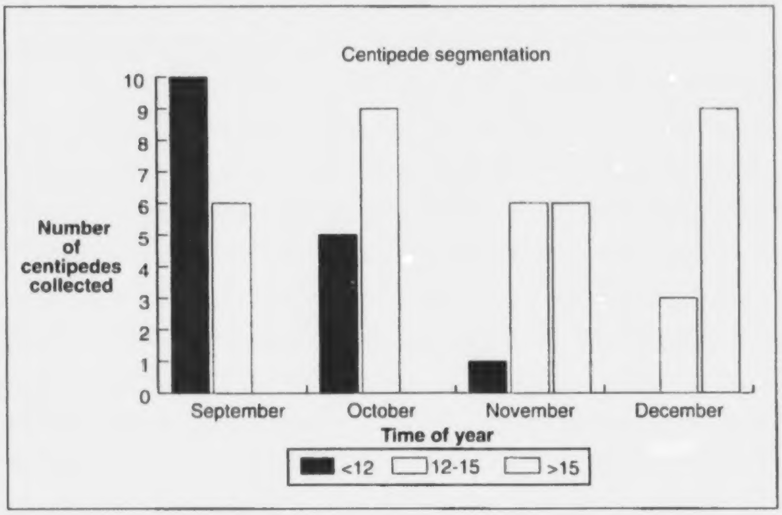
Collect soil-surface samples at various times during the year. Follow the sample procedure in the Core Experiment. Isolate the centipedes and determine the number of individuals of different ages by assuming that number of segments is correlated with age.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Hypothetical seasonal changes in the segmentation of a centipede population.

Month of collection	Number of centipedes in each size class		
	<12 Segments	12 to 15 Segments	>15 Segments
September	10	6	0
October	5	9	0
November	1	6	6
December	0	3	9



Graph C. Centipede segmentation over a four-month period.

Analyze these data for each size category by graphing the number of centipedes collected versus time of year. Advanced students can use Chi-Square Goodness of Fit Test to determine whether their results differ from a uniform distribution.

TEST QUESTION

If all members of one population within the community are similar in size and age, what does that tell you about the population?

Interpretation

Accept the hypothesis only if data show consistent decreases in the number of small individuals and consistent increases in the number of large individuals. The hypothesis should be rejected if all size or age categories are present during every sampling period.

Answer to Test Question

All members of the population belong to the same generation.

VARIATION 4

The Survival of Soil-Dwelling Organisms Following a Controlled Burn of Coastal Bermuda Grass

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from a Bermuda grass pasture before and after a controlled burn. They will evaluate the species diversity in both samples.

SAFETY PROCEDURE

The area should not be burned by the students. Fire departments and the park service do controlled burns. Any burn should be controlled carefully and should have been planned by one of these groups, not conducted solely for this exercise.

HYPOTHESIS GENERATION

Question

What effect does fire have on the organisms in the soil interface?

Sample Hypothesis

Fire will kill all the soil-surface organisms.

Rationale

Organisms cannot withstand the high temperatures and destruction of the fire's flames.

Sample Experimental Procedure

Collect soil-surface samples before and after a controlled burn of coastal Bermuda grass. Follow the sample procedure for the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Number of species present in soil-surface samples before and after a controlled burn of coastal Bermuda grass.

Common name of grouped species	Number of species present	
	Before burn	After burn
Annelid worms	1	1
Spiders	3	0
Mites	5	3
Centipedes	1	0
Millipedes	2	1
Springtails	5	1
Grasshoppers & crickets	1	0
Thrips	1	1
Beetles and weevils	3	2
Cockroaches	1	1
Ants, bees, and wasps	3	2

TEACHING TIP

Students can take additional samples over the next several months to measure the rates of recolonization for various taxa.

Interpretation

The hypothesis should be accepted if the kinds of species differ before and after the fire. No individuals from any species will be present. Reject the hypothesis if some of every species group survive. Some taxonomic groups may be more heat-tolerant than others. Some may be less susceptible because of their position in the soil strata.

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To analyze these data, calculate the total number of species collected before and after the burn. This number should be expressed as a percentage of the original density. Advanced students can use a diversity index to compare the samples. (See Core Experiment for instructions for calculating a diversity index.)

Answer to Test Question
60%

TEST QUESTION

Using the following information, what percent of mites survived after burning?

Table 7. Number of species present in humus samples before and after a controlled burn of coastal Bermuda grass.

Common name of grouped species	Number of species present	
	Before burn	**After burn**
Annelid worms	1	1
Spiders	3	0
Mites	5	3
Centipedes	1	0
Millipedes	2	1
Springtails	3	1

VARIATION 5

The Arthropod Fauna in Different Types of Compost

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from two compost piles, one containing mostly grass and the other containing mostly leaves. They will identify and count all representatives of major taxa.

HYPOTHESIS GENERATION

Question

Is there a difference in the number of organisms found between compost piles with leaves and ones with grass growing?

Sample Hypothesis

The relative abundance of animal species will be lower in samples from the compost pile containing mostly grass than in the compost pile of leaves.

Rationale

There will be fewer organisms in the compost pile with grass, as there will be less decaying matter as an energy source.

Sample Experimental Procedure

Collect samples of several types of compost. These types may include compost from leaves, grass clippings, vegetable scraps, or a mixture of leaves, clippings and vegetables. Follow the sample procedure for the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 8. Number of individuals in samples collected from several types of compost.

Common name of grouped species	Number of individuals present	
	Leaf-rich compost	Grass-rich compost
Annelid worms	4	6
Spiders	6	8
Mites	18	4
Sowbugs	10	8
Centipedes	3	7
Millipedes	6	6
Symphylans	15	9
Springtails	57	43
Thrips	12	5
Ants	10	4
Beetles	7	3

Analyze these data by ranking the identified arthropods by abundance. Determine which groups are most and least abundant and draw pie charts showing the relative abundance of each group. Advanced students can also use a diversity index to compare samples.

Interpretation

Accept the hypothesis if there is a difference in the rank order of the groups between compost piles. Reject the hypothesis if the ranking is similar for all types of compost.

TEST QUESTION

Which variable is LEAST likely to affect the abundance of insects in a compost pile?

A. Moisture content
B. Distance to woods
C. Temperature
D. Predators

Answer to Test Question

B

VARIATION 6

The Density of Small Annelids in Soils with Different Moisture Contents

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will collect samples from sites with different percent moisture content and process in a Berlese-Tullgren. They will count all collected enchytraeid worms.

TEACHING TIP

Let students discover on their own that soils with 30 to 85% moisture will have fewer worms than soils with 0 to 20% moisture (Wallwork, 1976).

HYPOTHESIS GENERATION

Question

How does moisture content of soil affect worm population?

Sample Hypothesis

There will be more worms in moist soil than in dry soil.

TEACHER'S NOTES

Rationale

Worms need moisture. Without it, they dehydrate and die. Therefore, worms should be found in higher density in moist soils.

Sample Experimental Procedure

Choose three sample collection sites that appear to have consistently different moisture content, such as a frequently watered lawn area and a lawn not well watered. Collect two samples from each site. One sample from each site will be treated according to the procedure from the Core Experiment. The second sample from each site will be examined for its moisture content. The moisture content can be determined by first massing the sample, and then drying it in an oven at 100°C overnight before massing again. Use the following equation to calculate the moisture content:

$$\frac{\textit{Mass of Dried Sample}}{\textit{Mass of Undried Sample}} \times 100\% = \%\ \textit{Moisture Content}$$

DATA ANALYSIS AND INTERPRETATION

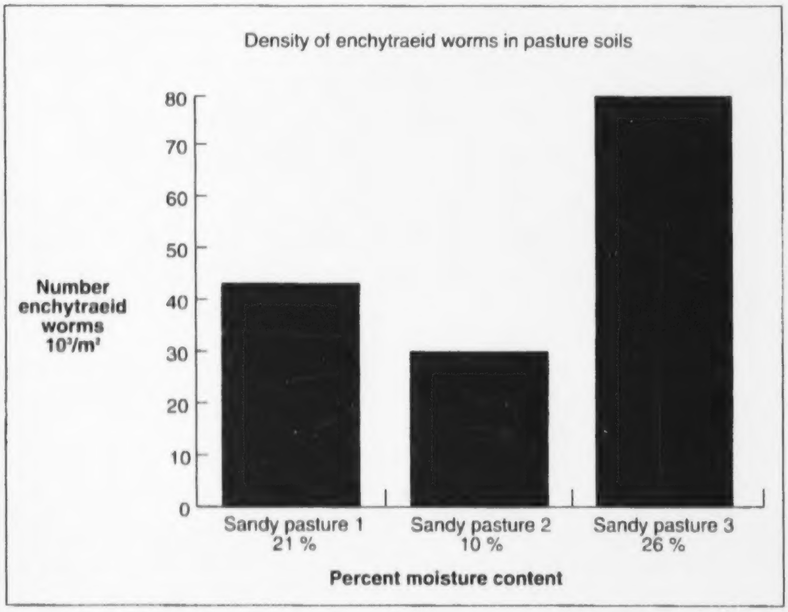
Sample Data

Table 9. Density of enchytraeid worms in pasture soils. The worm density data are summarized from the literature in Wallwork (1970). The moisture content values are hypothetical.

Site	Number of enchytraeid worms ($10^3/m^2$)	Percent moisture content
Sandy pasture 1	44	21
Sandy pasture 2	30	10
Sandy pasture 3	74	26

Interpretation

If the number of worms increases with increasing soil moisture, accept the hypothesis. If the number of worms does not increase with increasing soil moisture, reject the hypothesis.



Graph D. Enchytraeid worm density.

TEST QUESTION

How would you use soil moisture data to find the best site for collecting fishing worms if large fishing worms are influenced by soil moisture the same way enchytraeid worms are influenced?

Answer to Test Question

One would select soils with high moisture content over sites with lower moisture content because more earthworms are in moist soil.

VARIATION 7

The Soil pH and Diversity of Soil Organisms

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from sites with different hydrogen ion concentrations. They will identify, classify, and count all organisms collected.

MATERIALS NEEDED

1 vial Hydrion paper (pH 1 to 12)

HYPOTHESIS GENERATION

Question

How does soil pH affect species diversity?

Sample Hypothesis

Species diversity will decrease as the pH diverges from the neutral pH 7.

Rationale

Organisms survive within specific pH ranges. A neutral pH is optimum. Variance from this would be injurious to the organism.

Sample Experimental Procedure

Test the pH of soil from sites that you expect might have a different pH. Test the soil pH by mixing a match-head sized chunk of soil with about the same volume of water to make a paste. Use Hydrion paper to determine the approximate pH. Collect samples from soils of high, low, and neutral pH. Treat them according to the instructions for the Core Experiment.

TEACHING TIP

Acidic sites are likely to be found under pines or where acid rain is a problem. Basic sites are likely to be found in areas used by animals, lime-treated soils, or near lime outcroppings.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 10. Number of soil organisms from soils of different pH values. The organism values are approximated from data summarized from the literature in Wallwork (1970). The pH values are hypothetical.

Common name of grouped species	**Number of individuals collected per m^2 from:**		
	Limestone grassland (pH 8.2)	**Moor (pH 7.8)**	**Grassland (pH 6.9)**
Ticks and mites	45,300	43,000	78,800
Collembola	77,900	38,900	55,900
Enchytraeid worms	18,500	195,000	123,000
Lumbricid worms	389	0	11

Calculate Simpson's Diversity Index. See the Core Experiment for directions.

Interpretation

Each group of organisms tends to have a pH that is specific to their survival.

TEST QUESTION

How does water pH affect diversity?

Answer to Test Question

Answers will vary with student creativity. They could search for bodies of water with different acidity or take water from one site and observe changes after altering the pH.

VARIATION 8

Carnivores and Herbivores in the Schoolyard

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will determine the number of herbivores and carnivores in the schoolyard. Based on these findings, they will determine whether herbivores' or carnivores' survival is more adapted to this environment.

HYPOTHESIS GENERATION

Question

Are more herbivores or carnivores found in schoolyard soil surface?

Sample Hypothesis

More herbivores than carnivores will be found in a soil-surface sample collected from school grounds.

Rationale

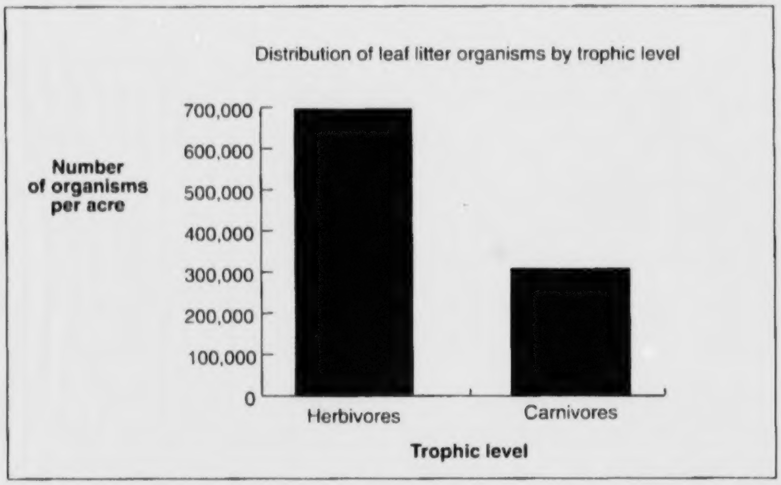
Students should derive their own rationale for the hypothesis.

Sample Experimental Procedure

1. Collect soil-surface samples from the school ground.
2. Follow the sample procedure from the Core Experiment.
3. Determine how many of these organisms were herbivores or carnivores.

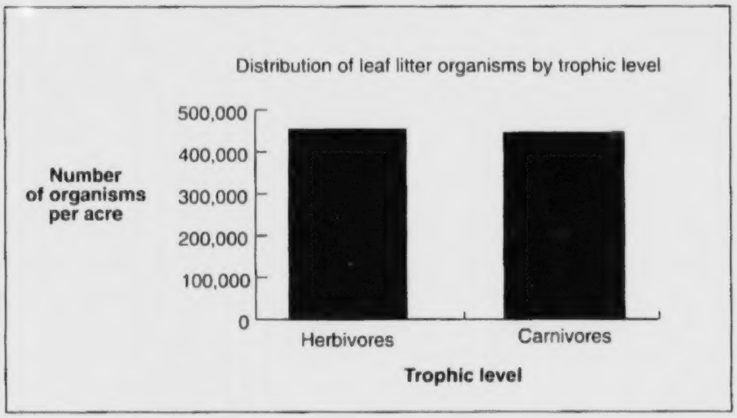
DATA ANALYSIS AND INTERPRETATION

Sample Data 1



Graph E. The number of soil organisms by diet collected per acre from leaf litter of a wet bottom area during spring.

Sample Data 2



Graph F. The number of soil organisms by diet collected per acre from leaf litter of a wet bottom area during spring.

TEST QUESTION

Examine the two graphs above. Account for the different ratios of herbivores to carnivores in the two communities.

Interpretation

More herbivores than carnivores were found in the sample of humus from the schoolyard, so the hypothesis is supported.

Interpretation

The same number of herbivores and carnivores were found in the sample of humus from the schoolyard, so the hypothesis is not supported.

Answer to Test Question

One would expect more herbivores than carnivores in a community because only 10% of the energy available to the herbivore is available to the carnivore that consumes it. This is based on the assumption that herbivores are smaller than carnivores. The second set of data, however, shows equal numbers of herbivores and carnivores—something unexpected. This may be explained by a larger biomass for individual species of herbivores than their carnivore predators. Also, the carnivores may consume other organisms such as decomposers, thus giving them an alternative food source. In any case, the carnivores will receive only 10% of the energy originally available to their prey.

VARIATION 9

Organism Size Distribution

TEACHING TIPS

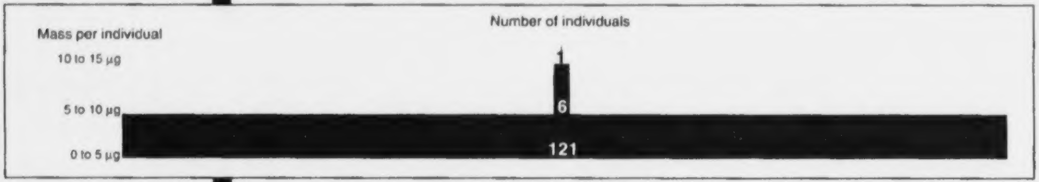
- Results are likely to be different in soils of different textures.
- A calibrated ocular micrometer will facilitate sizing.
- Biomass also can be used to assess size. Although biomass values, such as those shown in Table 11, are more common in the literature, they will be very difficult for students to collect.

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from one site. They will count the number of organisms in each of several sizes of classes determined by body length.

Table 11. This pyramid of numbers, constructed from data presented in Engelmann (1961), shows the inverse relationship between body size and population density.



HYPOTHESIS GENERATION

Question

Is there a relationship between an organism's size and the number of individuals that will be found in a sample?

Sample Hypothesis

The number of individuals will increase as their size decreases.

Rationale

The smaller in size the organism, the greater the number of this species found in the sample.

Sample Experimental Procedure

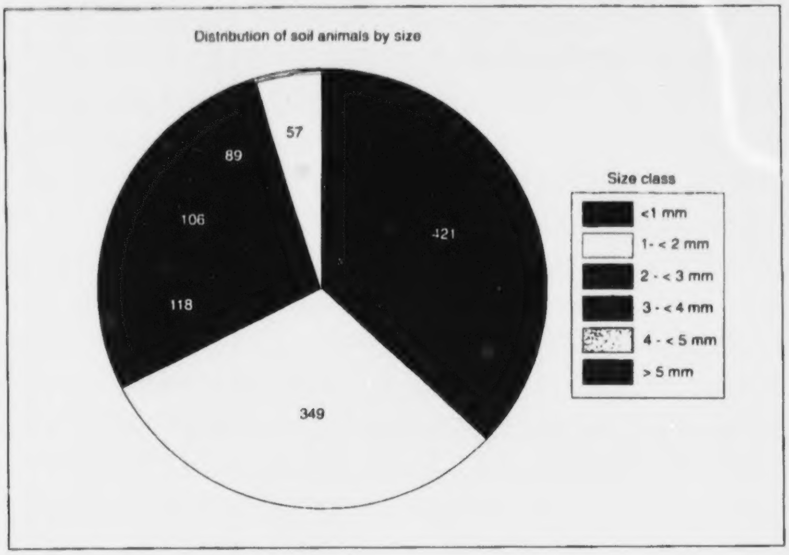
Collect a sample from forest or grassland soils and treat it according to the instructions for the Core Experiment. Establish size classes at millimeter intervals for organisms less than 5 mm long.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 12. Distribution of soil animals by size. These data are for cryptozoa from South Africa forest soils (Birch & Clark, 1953).

Size class	Number of organisms
<1 mm	421
1-<2 mm	349
2-<3 mm	118
3-<4 mm	106
4-<5 mm	89
>5 mm	57



Graph G. Distribution of soil animals by size.

TEST QUESTION

Design an experiment to determine whether the size distribution of soil organisms varies with soil texture.

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Interpretation

Accept the hypothesis if the number of organisms decreases with increasing size. Reject the hypothesis if there are more organisms in the larger size classes.

Answer to Test Question

Answers will vary, but should include sampling from at least two sites, analyzing the texture of the soil and counting organisms by size class.

A Community Underfoot: Density and Diversity of Invertebrates in Soil or Ground Cover

Directions for Students

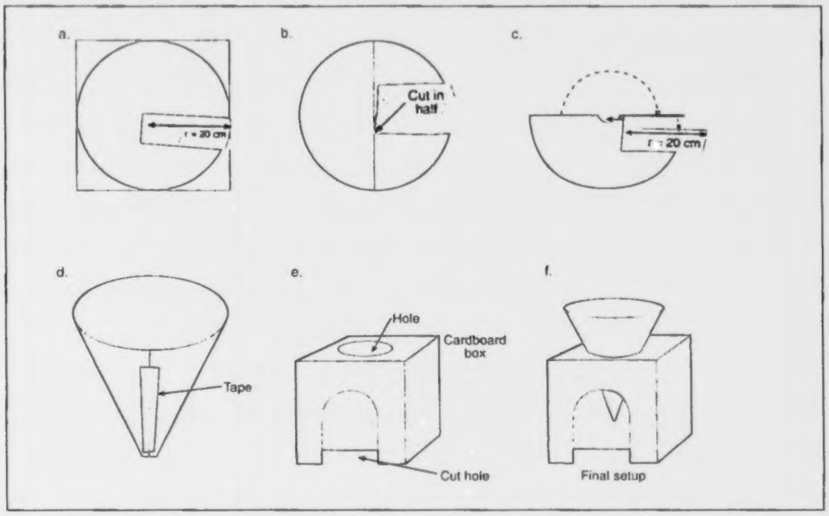

Figure 1. Extraction funnel assembly.

INTRODUCTION

Have you ever wondered what type of life exists beneath your feet? What do the organisms look like? How big are they? What do they eat? Do they benefit humans? You will explore the cryptic creatures of the below-ground world as you create and use a sampling device, learn to identify them, and construct simple trophic pyramids with your data.

OBJECTIVES

At the end of this lab, you should be able to:

- Construct a Berlese-Tullgren funnel to sample invertebrate fauna in soil or ground cover samples.
- Estimate the population density and diversity for different environmental conditions.
- Describe the effects of specific environmental variables on a target population or community structure.

SAFETY PROCEDURES

Wear gloves whenever handling soil samples and wash your hands at the beinning and end of the lab.

Do not use alcohol near an open flame, as it is flammable.

Do not drink the alcohol or inhale its fumes.

Have adequate ventilation in the classroom.

Reduce vapor levels by using a collecting jar that fits snugly with the funnel. Keep collecting jars and petri dishes covered.

Keep the 25-watt bulb at least 15 cm away from the larger Berlese-Tullgren funnel sample to prevent the ignition of any dry leaves.

Disturb the soil-surface as little as possible when collecting samples.

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MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 3 2-L plastic soda bottles
- 1 pair scissors
- 3 10 x 10 x 5-cm soil-surface samples
- 1 pair of gloves
- 3 1-L (1-quart) sealable, plastic bags
- 3 10-cm^2 square 1/4 or 1/8-inch mesh hardware cloth or plastic needlepoint backing
- 3 500-mL glass jars with 7.5-cm diameter openings with tight lids
- 3 9-watt colorless light bulbs and 3 1-socket sections of a string of holiday lights or 3 25-watt shielded lights
- 1 stereoscope
- 1 eyedropper
- 1 forceps
- 1 9 x 14-cm (8.5 x 11-inch) sheet each of black and white construction paper
- 3 10-cm^2 square of aluminum foil or dark paper
- 5 sheets of newspaper
- 150.0 mL 70% isopropyl alcohol
- 1 garden trowel

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of soil organisms and ecology. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis.

Answer the following questions:
1. What is the question you are investigating?
2. Why is controlling variables important?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:
1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:
1. Construct a bar graph showing kinds of organisms collected and their abundance.
2. Do your data support or refute your hypothesis?
3. Using your specific data, explain your answer to Question #2.

4. Compare your data with that of other groups. Are these data the same or different? Why or why not?
5. Why is it necessary to have repeated sampling?
6. Will the number or kinds of organisms found in a sample always be the same?
7. What could cause the difference? Be specific.
8. What did you learn from this activity?
9. Make a statement that represents what you learned about animal diversity in the soil.

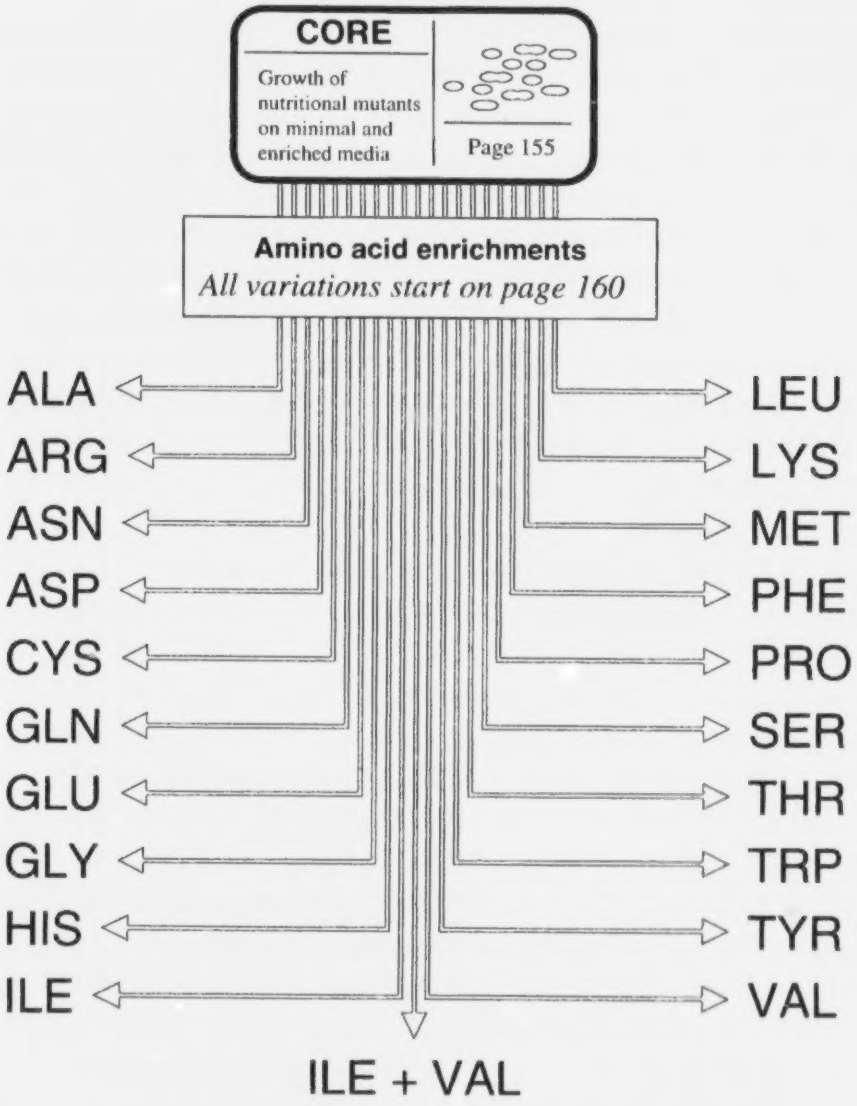
DESIGN OF VARIATIONS OF CORE EXPERIMENT

After collecting and analyzing these data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for experiments you could do next. Think of questions that occurred to you as you conducted the Core Experiment on organisms found in the soil surface. Design an experiment that is quantifiable and write your procedure in a numbered list of steps. Questions other students have studied include the following:

- How will cattle grazing affect density of mites in a pasture?
- Is millipede density affected by exposure to north- or south-facing slopes?
- Is the age of a generation of centipedes similar?
- What effect does fire have on the organisms in the soil interface?
- Is there a difference in the number of organisms found between compost piles with leaves and ones with grass growing?
- How does moisture content of soil affect worm population?
- How does soil pH affect species diversity?
- Are more herbivores or carnivores found in schoolyard soil surface?
- Is there a relationship between an organism's size and the number of individuals that will be found in a sample?

Isolation of Nutritional Mutants of *E. coli*

At-A-Glance Map of the Core Experiment and Its Variations


CORE
Growth of nutritional mutants on minimal and enriched media
Page 155
Amino acid enrichments
All variations start on page 160
ALA
ARG
ASN
ASP
CYS
GLN
GLU
GLY
HIS
ILE
LEU
LYS
MET
PHE
PRO
SER
THR
TRP
TYR
VAL
ILE + VAL

NOTES

Isolation of Nutritional Mutants of *E. coli*

For the safe implementation of this lab, consult *Working with DNA & Bacteria in Precollege Science Classrooms* (1993). Toby Mogollon Horn. NABT: Reston, VA.

SYNOPSIS FOR CORE EXPERIMENT

Students will select individual colonies of *Escherichia coli* (*E. coli*) bacteria and design an investigation that demonstrates genetic variation to determine if any individual colonies are nutritional mutants.

APPROPRIATE BIOLOGY LEVEL

Advanced

TEACHER PARTNERS

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SCIENTIST PARTNER

Dr. Kenneth Kreuzer
Duke University Medical Center
Microbiology Department
Box 3020
Durham, NC 27710

Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 160. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Explain that bacterial species show genetic variations.
- Design an experiment to differentiate between mutant and wild-type *E. coli* bacteria.
- Explain the relationship between an individual bacterium and a colony.

MATERIALS NEEDED

For the teacher preparation, you will need the following for a class of 24:

1 bottle antibacterial soap

1 BIO 101, Inc. DNA Library Media Kit, Catalog # 5800-100 (see Teaching Tips, p. 154)

OR

the components of the kit:

Rich Luria Plates

- 10.0 g tryptone
- 5.0 g yeast
- 0.5 g NaCl
- 15.0 g agar

Minimal Plates

Part A
- 10.0 g glucose
- 0.12 g $MgSO_4$
- 0.011 g $CaCl_2$
- 1.0 mg thiamine

Part B
- 15.0 g granular agar

Part C
- 7.0 g K_2HPO_4
- 3.0 g KH_2PO_4
- 0.5 g NaCl
- 1.0 g NH_4Cl

Part D
- 0.135 g $FeCl_3 \cdot 6\,H_2O$
- 0.010 g $MnCl_2 \cdot 4\,H_2O$
- 0.5 mL 1M HCl
- 1 20-mL sterile vial
- 1 0.22-µm sterile millipore filter
- 1 10-cc sterile disposable syringe

(continued on p. 150)

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (30 minutes)
- Introduce and set up the lab.

Day 2 (45 minutes)
- Conduct the lab.

Day 3 (15 minutes)
- Observation of results and discussion.

PREPARATION TIME REQUIRED

30 minutes

- Order and locate materials.

Media

2 hours

- Prepare and autoclave media and pour plates with BIO 101 media kit. Prepare bleach solutions.

OR

5 hours

- Prepare media with recipes found in Directions for Setting Up the Lab.

30 minutes

- Pour and label plates.

Library Plates

30 minutes

- Streak plates with original library culture.

(continued from p. 149)

- 3.0 L warm distilled H_2O
- 2 2-L Erlenmeyer flasks
- 80 to 120 10-cm sterile petri dishes
- 1 autoclave or pressure cooker
- 1 autoclave marking pen
- 1 100-mL Erlenmeyer flask
- 1 150-mL Erlenmeyer flask
- 1 50-mL Erlenmeyer flask
- 1 1-L sterile Erlenmeyer flask
- 1 sterile pipette
- 1 refrigerator
- 80 to 120 sterile toothpicks or 1 nichrome wire loop *(optional)*
- 1 bunsen burner *(optional)*
- 350 mL household bleach
- 2 L distilled water
- 1 1.5-L container with lid
- 12 250-mL spray bottles

You will need the following for each group of two students in a class of 24:

- 1 bottle antibacterial soap
- 1 pair disposable gloves *(optional)*
- 1 spray bottle with 10% household bleach
- 100 mL 20% household bleach
- 1 250-mL beaker
- 2 photocopied grids
- 1 rich medium plate
- 1 minimal medium plate
- 1 library plate
- 1 permanent marker
- 60 sterile toothpicks
- 1 covered, sterile toothpick container
- 1 incubator *(optional)*

SAFETY PROCEDURES

Clean lab tables with 10% household bleach solution before and after use.

Wash hands thoroughly with warm water and antibacterial soap before and after lab.

Place all materials that have touched bacteria into a biohazard bag and autoclave. If autoclave is not available, sterilize with 20% household bleach solution for one hour before disposal. Check with local universities, community colleges, or veterinary offices for assistance with disposal of materials.

Check with Student Health to determine if there are any students in the class who have immune deficiency as a result of disease or chemotherapy. These persons should not be in the room where there is experimentation with live bacteria.

Exercise caution with clothing, hair, and other flammable materials while working near open flames.

Handle glass with care. Place broken glass in a designated container, not with the general trash.

Always wear goggles and aprons.

Tie back long hair and secure bulky clothing.

Wear nonallergenic, disposable gloves when plating bacteria.

DIRECTIONS FOR SETTING UP THE LAB

Prepare BIO 101 Kit as per enclosed instructions.

OR

If kit is not purchased, prepare rich and minimal media as follows:

Five days ahead

1. Prepare rich and minimal media plates.
2. Cool to room temperature. Leave at room temperature for 2 days. Then, refrigerate until needed.

Rich Plates

For 1 L of medium:

1. Combine the following in a 2-L Erlenmeyer flask:
   - 10.0 g tryptone
   - 5.0 g yeast extract
   - 0.5 g NaCl
   - 15.0 g agar
2. Add warm, distilled water to the components of Step 1. Bring to 1 L volume.
3. Autoclave for 15 minutes at 15 pounds per square inch (psi).
4. Pour into sterile, disposable or glass petri dishes and allow to solidify. Makes approximately 40 plates.

Minimal Plates

1. For 1 L of medium prepare the following parts and **autoclave separately** to avoid precipitation.

Part A. M9 Mix with Thiamine

a. Combine the following in a 100-mL Erlenmeyer flask:
- 10.0 g glucose
- 0.12 g $MgSO_4$
- 0.011 g $CaCl_2$
- 1.0 mg thiamine

b. Add components of Step "a" to 45 mL distilled water.
c. Dissolve with heat and stirring.
d. Bring to a total volume of 50 mL with distilled water.
e. Autoclave 15 minutes at 15 psi.

Part B. Agar-B

Combine the following in a 2-L Erlenmeyer flask:
a. Add 15 g granular agar to 850 mL warm, distilled water.
b. Autoclave 15 minutes at 15 psi.

Part C. 10x M9 Salts

a. Combine the following in a 150-mL Erlenmeyer flask:
- 7.0 g K_2HPO_4
- 3.0 g KH_2PO_4
- 0.5 g NaCl
- 1.0 g NH_4Cl

b. Add the components of Step "a" to 95 mL distilled water.
c. Dissolve with stirring.
d. Bring to a total volume of 100 mL with distilled water.
e. Autoclave 15 minutes at 15 psi.

Part D. Trace Minerals

a. Combine the following in a 50-mL Erlenmeyer flask:
- 0.135 g $FeCl_3 \cdot 6\,H_2O$
- 0.010 g $MnCl_2 \cdot 4\,H_2O$

b. Add components of Step "a" to 49.5 mL distilled water.
c. Add 0.5 mL 1M HCl.
d. Swirl to mix.
e. Filter sterilize the solution in a sterile 20-mL vial with a sterile 0.22-mm millipore filter and 10-cc sterile disposable syringe. See Figure 1 for sterilization setup.
f. Do **not** autoclave.

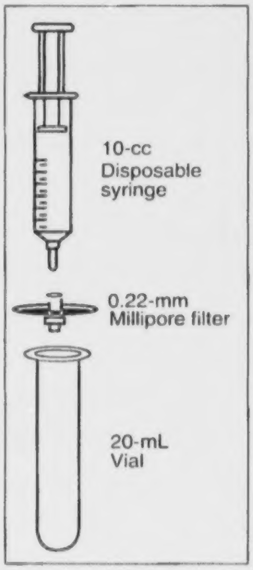

Figure 1. Sterilization setup.

TEACHER'S NOTES

TEACHER'S NOTES

2. When the Agar-B solution has cooled to approximately 55°C, the agar will be molten and still hot to the touch. Add the M9 Mix with Thiamine and the 10x M9 Salts Solution to a sterile 1-L Erlenmeyer flask.
3. Add 0.1 mL sterilized Trace Minerals solution with sterile pipette to the Erlenmeyer flask.
4. Gently swirl to combine.
5. Pour minimal medium into sterile petri dishes and allow to solidify. Makes approximately 40 plates.

Two days ahead

1. Remove plates from refrigerator and allow to come to room temperature.
2. Streak out a plate for every two students from the library culture to allow single colonies of bacteria to grow. See Figure 2. Plates also can be streaked, grown several days ahead, and then refrigerated. See Teaching Tips. Streaking is best done with a flame-sterilized nichrome wire loop, but sterile toothpicks may be used. There should be plenty of single, isolated colonies on the streaked plates for students to select easily.

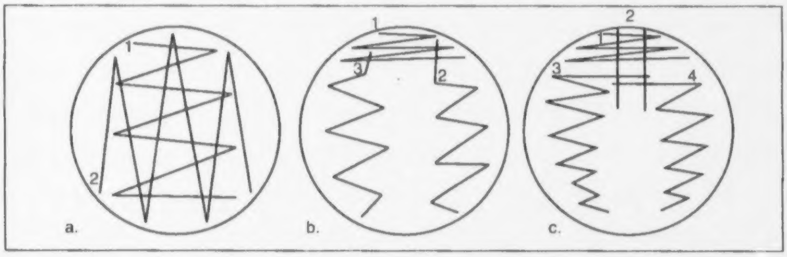

Figure 2. Suggested patterns for streaking plates. Dip the sterile loop or toothpick into the library. Then, streak the horizontal line 1 as shown. Resterilize the loop or use a fresh toothpick *without* dipping into the library for each additional streak.

3. If you find that colonies are too dense on the plate despite using the suggested streaking patterns, dilute the library 1:10 in sterile liquid medium, i.e. plate medium without agar, before streaking.

One day ahead

1. Prepare grids. See Figure 3 for grid.

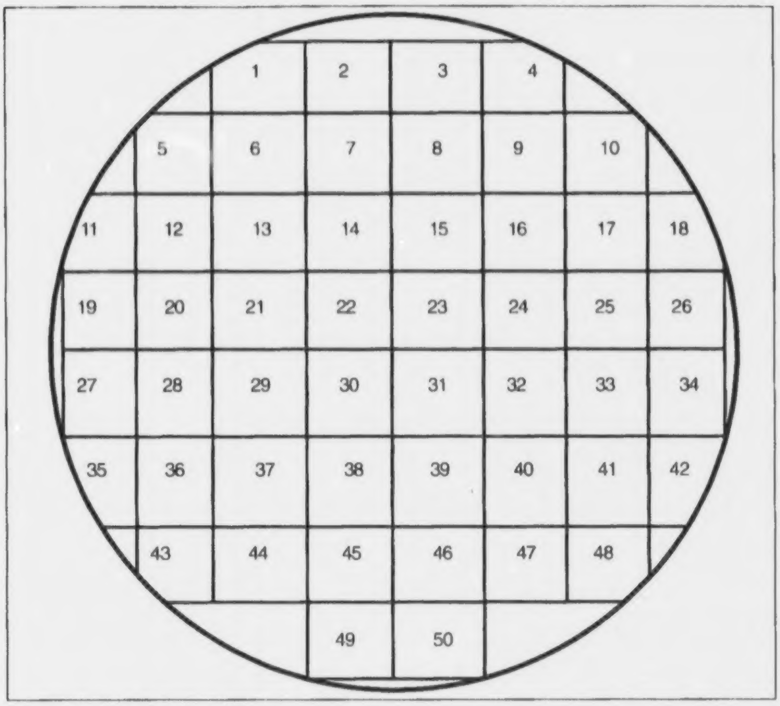

Figure 3. Grid for petri dishes.

2. Prepare 20% bleach solution. Add 200 mL household bleach to 800 mL distilled water. Store in a 1.5-L container with lid, and cap tightly.
3. Prepare 10% bleach solution. Add 150 mL household bleach to 1350 mL distilled water. Dispense 100 mL into each of 12 250-mL spray bottles.

TEACHER BACKGROUND

Content Information

Escherichia coli (*E. coli*), a bacterium that occurs naturally in the human intestine, is used frequently for bacteriology and genetic engineering experiments. The strain used in this experiment is not pathogenic and cannot cause any disease. However, students still need to use sterile technique to keep their plates free of contaminants. They should be taught the correct procedures for safely working with bacteria in the laboratory.

The *E. coli* used in this lab was made from a common laboratory strain identified as N1624. The bacteria were mutated with transposons, introduced by infecting the cells with a special strain of lambda bacteriophage. This process creates a *library* of millions of bacterial cells, each with a different mutation. Transposons or transposable elements are typically small [500 to 10,000 base pairs (bp)] pieces of DNA that can excise themselves from one position in a prokaryote's genome and insert themselves into a different place in the same genome or into a different organism's genome (Gardner et al., 1991). See Figure 4. There is often little or no specificity for the sites where the transposon integrates into the target chromosome. Typically, transposon insertion into a gene prevents that gene's expression. See Figure 4. Every bacterial cell in a transposon-mutated library contains one transposon inserted somewhere in its genome.

TEACHER'S NOTES

TEACHING TIPS

- The library of nutritional mutants is available at the cost of shipping, approximately $10, from:

  Dr. Ken Kreuzer
  Microbiology Department
  Box 3020
  Duke University Medical Center
  Durham, NC 27710
  919.684.6466
  Email:
  Kenneth.Kreuzer@Duke.edu
- Minimal medium and rich medium, Luria Broth, can be purchased from science supply houses. BIO 101, Inc., 1070 Joshua Way, Vista, CA 92083; phone 800.424.6101; fax 619.598.0116 sells the medium necessary for this lab in a kit identified as DNA Library Catalog #5800-100. Scratch recipes and directions for media are listed in Directions for Setting Up the Lab (pages 150 to 152).
- Autoclave Parts A, B, and C of the Minimal Media in separate flasks to avoid precipitation.
- Media must be autoclaved before pouring into sterile petri plates. For volumes one liter or less per container, 15 minutes at 15 pounds per square inch (psi) of pressure at 121°C is sufficient. A pressure cooker at 15 psi for 15 minutes can serve as an alternative for the autoclave. Be sure to fill the container only half full and cover the top loosely with a cotton plug or foil. Hospitals, veterinarians, doctors, or universities may have autoclaves and are often willing to sterilize materials.
- Plastic sterile petri dishes are disposed of easily in 20% household bleach solution or by autoclaving. Glass petri dishes must be sterilized before and after use. You also may use prepoured plates, but they are expensive.
- Teach students sterile technique, also known as aseptic technique, prior to this lab.
- Have students observe *E. coli* with either prepared slides or slides they prepare themselves.
- Explain that the bottoms of petri dishes should be labeled as they contain the material of interest, not the petri dish tops that can be interchanged.

(continued on page 155)

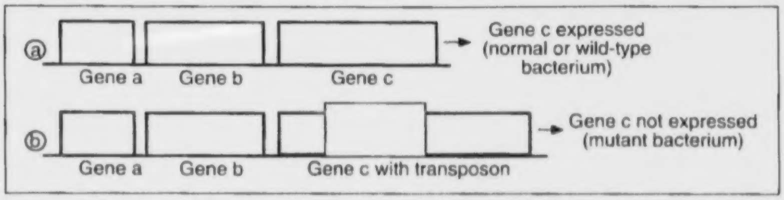
ⓐ
Gene a
Gene b
Gene c
Gene c expressed (normal or wild-type bacterium)
ⓑ
Gene a
Gene b
Gene c with transposon
Gene c not expressed (mutant bacterium)

Figure 4. The effects of transposon insertion on gene expression.

Every protein is made up of combinations of 20 amino acids. Wild-type *E. coli* cells produce all 20 common amino acids. This allows the cells to produce all the proteins necessary for growth. Enzymes are proteins that catalyze the conversion of one chemical into another. Each of the 20 amino acids is synthesized by a series of enzymes. The enzymes necessary for producing one amino acid are generally different from those needed to produce a different amino acid. These enzymes for amino acid biosynthesis are each encoded by different genes in the bacterial chromosome. For example, the enzymes for synthesizing the amino acid histidine are encoded by the *his* genes. If the bacterial cell contains a mutation, such as an inserted transposon in one of the genes necessary for synthesizing histidine, this cell and its descendants will be unable to grow unless histidine is supplied. This kind of mutant is known as a nutritional mutant. A mutant requiring histidine for growth would be known as a histidine nutritional mutant.

The library of *E. coli* cells contains a collection of millions of different transposon insertion mutants with the transposon inserted randomly into the nonessential genes of the *E. coli* chromosome. If a transposon is inserted into an essential gene, the mutant cannot grow and therefore cannot be isolated. Every colony that grows on the original streak plate will be a different mutant with one transposon somewhere in its chromosome. However, because most bacterial genes are not necessary for amino acid biosynthesis, most of the mutants are not nutritional mutants. In this library, roughly one out of every 200 colonies has been found to be a nutritional mutant. See Figure 5. Usually, each nutritional mutant fails to produce only a single amino acid. On the other hand, the chemical structures of isoleucine and valine are similar. The pathways for synthesizing these amino acids share four of the same enzymes. A mutant defective in one of these enzymes will require both isoleucine and valine to grow.

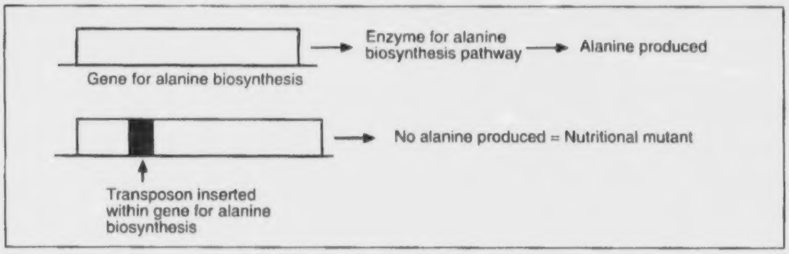
Gene for alanine biosynthesis
Enzyme for alanine biosynthesis pathway
Alanine produced
No alanine produced = Nutritional mutant
Transposon inserted within gene for alanine biosynthesis

Figure 5. Transposon blocking production of amino acid.

Bacteria reproduce by fission. See Figure 6. A single cell forms a colony of genetically identical cells. *E. coli* cells divide every 20 minutes if they have sufficient nutrients and ideal conditions. With simple calculations, students can determine how many cells a single *E. coli* can produce in 24 hours. *E. coli* grow most rapidly at 37°C. They will grow at room temperature, but it may take two days to obtain visible, isolated colonies.

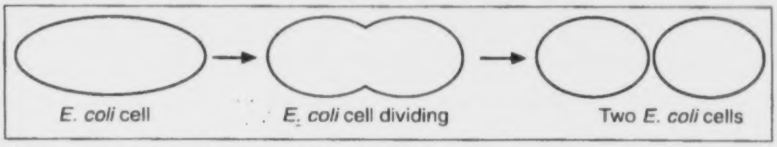

Figure 6. *E. coli* bacterium reproducing by fission.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

Correct Concept	Misconception
• Individuals within a bacterial species can be genetically variable.	• All bacteria of one species are genetically identical.
• Bacteria, like all organisms, have certain nutritional requirements.	• Bacteria can grow on anything.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Describe how the library was prepared, as described in the Teacher Background. Each team will receive a streaked plate that contains a sample from the library. Point out individual colonies on the plates and stress the fact that each grew from a single bacterial cell. They can expect about one in 200 colonies to be a nutritional mutant identifiable by its differential growth on rich and minimal media.

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

Explain that nutritionally mutant bacteria will not grow on minimal medium, but will grow on rich medium.

Sample Hypothesis

If bacteria do not grow on minimal medium, they are nutritionally mutant.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 159.

Question

Will bacteria that are not nutritional mutants grow on minimal medium and enriched medium?

Hypothesis

Bacteria that are not nutritional mutants will grow on the minimal medium but not as well as on the enriched medium.

Rationale

The minimal medium has a smaller quantity of the nutrients, such as amino acids, that allow these bacteria to grow rapidly.

Procedure

1. Wash hands with antibacterial soap and then put on disposable gloves.
2. Wipe down the lab bench with 10% household bleach.
3. Add 100 mL 20% bleach solution to a 250-mL beaker. Sterilize used toothpicks.

TEACHING TIPS

(continued from page 154)

- The size of the autoclave will determine how much medium can be sterilized. One run usually takes 50 to 60 minutes.
- Demonstrate how to use the grid with an overhead projector and show what a single colony looks like.
- Agar may be colored with food coloring if you think the minimal and rich media plates may be confused by your students, or simply mark the plate edges with different colors of magic markers to indicate the type of medium they contain.
- For every two students, the teacher should streak out a plate from the library culture before the lab to allow single colonies of bacteria to grow. Good-sized, single colonies will grow in 24 to 48 hours at room temperature or overnight if incubated at 37°C. If you grow the colonies at 37°C, put the plates in an incubator at the end of the day and remove them in the morning to prevent colonies from growing too large. The bacterial colonies may be kept in the refrigerator for several days. Having single colonies is essential for good results. Each pair of students needs at least 50 single colonies. Therefore, it is important to perfect your streaking technique ahead of time to insure you have enough single colonies.
- Put amino acids in small salt/pepper shakers.
- Sterilize toothpicks. Place at least 60 toothpicks into a petri dish for each pair of students. Autoclave 20 minutes at 15 psi.
- Incubate plates upside down.

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TEACHER'S NOTES

4. Obtain two photocopied grids. Place one grid behind the rich medium plate and the other grid behind the minimal medium plate.
5. Mark the top of each grid and the side wall of the top and bottom of each plate with a permanent marker to show alignment of the grid with the petri dish. In addition, write your initials and the date on the bottom of each petri dish. See Figure 7.

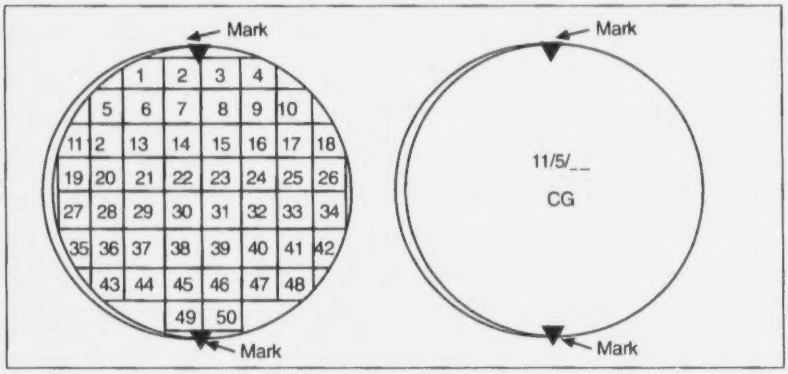


Figure 7. Labeling of petri dish.

6. Transfer 50 colonies from the library culture to one rich and one minimal medium plate in this way:
   a. *Lightly* touch a colony on the library culture with a sterile toothpick. You should not be able to see any residue on the toothpick. Be assured that you have plenty of bacteria as long as you touch the colony lightly. If you pick up too many cells, you may not have a good selection on minimal medium. Therefore, mutant colonies will be able to grow somewhat, and will be mistaken for wild-type.
   b. Touch the same toothpick to a space on the grid on a minimal medium plate and then to the corresponding space in the grid of the rich medium plate. See Figure 8.

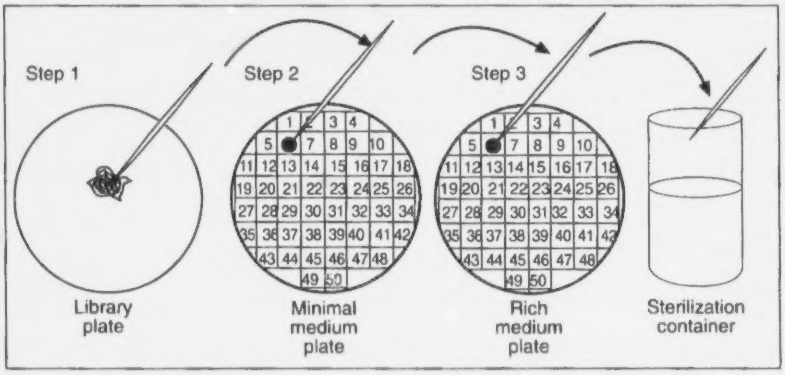


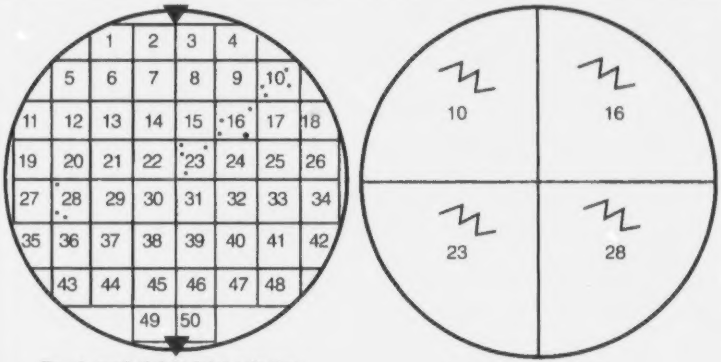
Figure 8. Transferring cultures from the library to the minimal and rich media plates.

   c. Dispose of the toothpick in the beaker with 20% household bleach solution.
   d. Repeat Steps 6a and 6b for each of the 50 colonies using a *new* toothpick and a *different* space on the grid for each colony.

7. Invert each petri dish and incubate overnight at 37°C, or in the room 2 days at 25°C.

8. When bacterial growth is evident, place the petri dishes over your grids. Align the marks and observe which colonies grew on rich medium versus minimal medium. Growth of a colony on rich medium, but no growth of that colony on minimal medium, indicates a mutant. The rich medium plate serves as a control, but the colony from the rich medium plate can be tested again on minimal medium to verify the results.

9. *(optional)* To verify your results and perhaps find more mutants, take any colonies that appear faint on the original minimal plate and re-streak them onto a second minimal plate. Touch a sterile toothpick to each colony on the first minimal plate and gently streak it 1 to 2 cm on a second minimal plate. Label each streak on the back of the plate or divide the plate into labeled sectors before streaking. You may find that some of the colonies will not grow on the second plate; they are probably nutritional mutants as well. Also, re-streak 1 or 2 positive controls, that is, colonies that grew normally on the original minimal plate. See Figure 9.



Touch toothpick to faint colonies on original minimal plate

Second minimal plate

Figure 9. The spots in boxes 10, 16, 23, and 28 represent poor growth of the bacteria. It may be a single colony in each box. All other boxes will have stong growth. Streak faint colonies 10, 16, 23, and 28 found on the original plate onto a second minimal plate.

DATA ANALYSIS AND INTERPRETATION

Sample Data 1

Five colonies, A through E (see Table 1) were selected from 1100 colonies streaked from a bacterial library. The library had an expected frequency of nutritional mutants of roughly 1:200. Of these colonies, A through C grew on the rich medium, but not on the minimal medium. Colony D grew on the minimal, but not the rich medium. E grew on neither the rich nor the minimal medium.

Colony	Growth on rich medium	Growth on minimal medium
A	+	-
B	+	-
C	+	-
D	-	+
E	-	-

Table 1. Colony growth from bacterial library.

Sample Data 2

Of 450 colonies from a bacterial library, five did not grow on minimal medium. These five were plated on 20 different plates, and each contained one of the 20 different amino acids. Number 1 grew on the glycine plate, #2 still did not grow on any of the plates, #3 and #4 grew on the cysteine plate, #5 grew on the asparagine plate and on the aspartic acid plate. Number 2 mutant was replated on isoleucine/valine, but still did not grow.

TEACHER'S NOTES

Interpretation

A through C are nutritional mutants deficient in some component provided in the rich medium that is not present in minimal medium. Colonies D and E probably were not transferred correctly since both grew on the rich medium master plate. Thus, the rate of nutritional mutation in the library was 3/1100 or 0.27%, or about half the expected rate of 0.5% (1/200).

Interpretation

Mutant #2 must require a vitamin, nuclei acid base, or other combination of amino acids.

TEST QUESTIONS

1. You have a subset of a library containing *E. coli* mutants that fails to grow at the temperatures of 37°C to 42°C which are best for wild-type growth, but grow as fast as wild-type at 20°C to 25°C. Design a procedure for isolating these temperature-sensitive mutants.
2. Why may you have to sample hundreds of colonies before finding a nutritional mutant?

Answers to Test Questions

1. Select individual colonies to duplicate plates. Medium type will make a difference. Incubate one plate at 42°C, the other at 20°C to 25°C. Colonies that grow on the low-temperature plate, but do not grow on the high-temperature plate, are temperature-sensitive mutants.
2. Bacteria contain many genes. Only a small fraction of them encode proteins that are involved in the synthesis of amino acids. The majority of the mutants in the library have a transposon inserted in a gene that is not necessary for amino acid biosynthesis.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed the data from the Core Experiment and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted the Core Experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- What did you find out in the Core Experiment?
- Why do you think some individual colonies grew on the minimal medium and some did not?
- What do the results indicate about different *E. coli* bacteria? Note to Teachers: Try to draw out the idea that an individual bacterium may be genetically different.
- If you found no growth on the minimal medium, what does this suggest about the differences in composition of the two media? Note to Teachers: If students suggest the minimal medium has less material, try to determine if they mean a smaller quantity of the same nutrients or fewer kinds of nutrients. At some point, you may need to inform students that the major difference between the two media is that the rich medium contains all 20 amino acids and the minimal medium lacks all 20 amino acids.
- Given that the major difference between the two types of media is the lack of the 20 amino acids in the minimal medium and their presence in the rich medium, why do you think some individual bacteria did not grow on the minimal medium. How could you test that idea?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p.15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

There are no modifications for this experiment that could make it acceptable to be performed by visually impaired persons.

Students who are blind or visually impaired should not be in a laboratory when experiments with bacteria are being performed. Having students who are not able to participate actively in a laboratory experiment is a concern, especially where the lab is small or a passageway is narrow. The possibility of accidentally bumping the arm of someone who is in the midst of working with live bacteria or upsetting equipment is too great.

Deaf or Hard-of-Hearing

Supply written instruction for every facet of this investigation. The instructor should see personally that the student has learned the proper techniques for transfer of bacterial colonies from plates to liquid or solid media, and that the student truly understands sterile technique.

Gifted

- Assign students to do a flow chart for the procedure as a collaborative learning experience.
- Ask students why the bacterial colonies stop growing if the bacteria can reproduce every 20 minutes.
- Have them calculate how many bacteria are in a single colony after a specific amount of time, given that bacteria can reproduce by fission every 20 minutes.
- Give students a chromosome map and have them identify the area on the chromosome where the transposon might have inserted to produce each mutant that they find.

Physically Impaired

Some colleges require students who are manually or mobility impaired to hire a person to perform their bacteriology lab experiments. Bacterial transfers are not done in a seated position.

One medical school built a frame to hold a wheelchair user in an erect position while having the experience of making a bacteriological culture and transfer.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. Nutritional mutants of *E. coli* can be found by:
    A. plating just on rich medium
    B. plating just on minimal medium
    C. plating on both minimal and rich media from the same colony.
2. The nutritional requirements of an *E. coli* mutant can be identified by:
    A. plating on minimal medium containing different amino acids
    B. plating on rich medium containing different amino acids
    C. plating on both minimal and rich media from the same colony.
3. Describe an experiment that would allow you to differentiate nutritional mutants from wild-type *E. coli*.

REFERENCES AND SUGGESTED READINGS

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Lacey, J.M. & Wilmore, D.W. (1990). Is glutamine a conditionally essential amino acid? *Nutrition Reviews, 48*(8), 297-306.

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Watson, J.D. & Crick, F.H.C. (1953). Molecular structure of nucleic acids. *Nature*, 171, 737-738.

Wickelgren, I. New genes for complete-protein beans. *Science News, 135*(19), 300.

POSSIBLE SOURCES OF MENTORS

American Society for Microbiology

United States Department of Agriculture

Answers to Additional Test Questions

1. C
2. A
3. Bacteria from a stock culture, called a library, would have to be streaked for isolation of single colonies. These colonies then could be picked out one at a time and plated first on minimal medium, then on rich medium with the same sterile toothpick. If bacterial growth forms on the rich medium plate but not on the minimal medium plate then you have discovered a mutant that must have a nutritional need not met in the minimal medium.

Answers to Questions and Analysis on Student Page

1. They may be at greater risk because many plants do not contain essential amino acids, such as methionine.
2. If there are only small amounts of these essential amino acids in the plant tissue, their absence forces the herbivore to eat great quantities of the plant or to seek these nutrients elsewhere.
3. Compare growth of mutants on a well-characterized source of all nutrients necessary for the wild-type organism versus the same source with nutrient supplements not normally required by wild-type.
4. Depending on the particular amino acid, *perhaps* some metabolic pathway converts the other amino acid into the missing one. If you found that a particular nutritional mutant can grow in the presence of either amino acid A or amino acid B, then you might suspect that one of these amino acids can be converted into the other. However, you could not tell which way the conversion occurs without more information. Therefore, the defect in the mutant might be in the synthesis of only one of the amino acids, but the other is able to "rescue" the growth of the mutant.
5. Bacteria unable to synthesize a specific amino acid could be streaked on medium containing food. If the bacteria grow, the food probably contains the amino acid in question.
6. Cooking destroys some amino acids. You could streak bacteria with known nutritional mutations on medium supplemented with extracts of food before and after cooking to see if there were any differences in their growth.
7. A transposon could insert into the genome and disrupt the function of a gene that normally rendered the bacterium sensitive to the antibiotic. More importantly, the transposons themselves cause drug resistance because they carry drug resistance genes.

VARIATIONS OF THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

VARIATIONS 1-21

Identification of Amino Acids Not Synthesized by Nutritional Mutants of *E. coli*

SYNOPSIS

Students will use minimal plates containing different amino acids to identify which amino acid(s) are not synthesized by each mutant.

ADDITIONAL MATERIALS NEEDED

For the teacher preparation, you will need the following for a class of 24:

- 20 different, sterile amino acid solutions (10 mg/mL; tyrosine at 2 mg/mL)
- 40 minimal medium plates
- 1 bent glass rod

You will need the following for each group of two students in a class of 24:

- 2 minimal medium plates
- 1 sterile amino acid solution
- 1 bent glass rod
- sterile filter paper disks *(optional)*

TEACHING TIPS

- It is best to use all 20 amino acids for this series of experiments to give students the best possible opportunity to identify mutants.
- Many amino acids can be purchased over the counter at drug stores in the vitamin section. Kits that contain all 20 amino acids may be purchased for around $70 to 90, or they may be bought separately from science supply houses. Three sources for all 20 amino acids are Sargent-Welch, Catalog #WL 4537D-60, 800.727.5229; Carolina Biological, Catalog #F6-84-3600, 800.334.5551; and Ward's, Catalog #38 W 6800, 800.962. 2660.
- Crystal Technique for Amino Acid Application
  1. Use sterile amino acids.
  2. Sterilize metal scoop by dipping in ethanol, heating in flame, dipping in amino acid, and sprinkling over the minimal medium.
  3. Allow to diffuse overnight.
- If you choose not to order sterile solutions of amino acids, you will need to make and sterilize them. Some amino acids should be filter-sterilized and others should be autoclaved, as indicated in Table 2. Preparation time will be approximately 4 hours if amino acid solutions are made and sterilized.

Autoclave	Filter
Alanine	Asparagine
Arginine	Aspartic Acid
Glutamine	Cysteine
Glutamic Acid	Tryptophan
Glycine	Tyrosine
Histidine	
Isoleucine	
Leucine	
Lycine	
Methionine	
Phenylalanine	
Proline	
Serine	
Threonine	
Valine	

Table 2. Sterilization method for amino acids.

- All 20 amino acids may be tested or a selection that is based on availability to the teacher.

(continued on page 161)

DIRECTIONS FOR SETTING UP THE EXPERIMENT

One day before

1. Spread 0.15 mL of each solution of 20 sterile amino acids onto 1 of 20 different plates of minimal medium using sterile technique. A bent glass rod should be used to spread the liquid amino acid solution. Alternately, you can apply a few crystals of amino acid powder to a minimal medium plate. Allow the crystals to diffuse into the minimal plates overnight.
2. Code each amino acid in the dish with the key found in tables, and label dishes appropriately.

HYPOTHESIS GENERATION

Question

Can the amino acid necessary for growth of a nutrient mutant be identified?

Sample Hypothesis

Most nutritional mutants require only one amino acid for growth.

Rationale

The amino acid deficiency of a nutritional mutant can be identified by growing the bacterium on plates containing each of the 20 different amino acids.

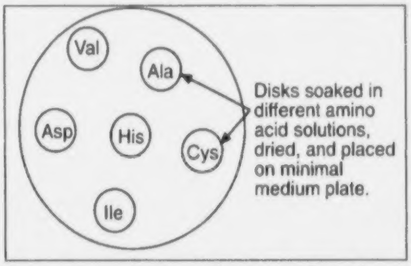
Val
Ala
Asp
His
Cys
Ile
Disks soaked in different amino acid solutions, dried, and placed on minimal medium plate.

Figure 10. Application of amino acids to plates.

Sample Experimental Procedure

1. After a mutant has been identified in the Core Experiment, have students transfer it from the rich medium plate to the minimal medium plate(s) containing the amino acid(s) to be tested.
2. The mutant should be streaked onto the plate and incubated as in the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Number	Amino Acid(s)	Number	Amino Acid(s)
1	Ala	11	Leu
2	Arg	12	Lys
3	Asn	13	Met
4	Asp	14	Phe
5	Cys	15	Pro
6	Gln	16	Ser
7	Glu	17	Thr
8	Gly	18	Trp
9	His	19	Tyr
10	Ile	20	Val
		21	Ile + Val

Table 3. Key for amino acids.

TEACHING TIPS

(continued from p. 160)

- An alternative method is to apply more than one amino acid to a single plate using small filter paper disks. A sterile filter paper disk can be soaked in the sterile amino acid solution and dried inside a sterile petri dish. See Figure 10. The petri dish lid should be slightly ajar. Store in a clean, undisturbed place such as inside a drawer. Several disks then can be applied to the surface of the agar on one minimal medium plate after streaking or spreading the *E. coli* mutant across the entire plate surface.

Interpretation

Mutant A appears not to be a nutritional mutant for any of the 20 amino acids. Mutants B and C are mutants for tryptophan; mutant D for cysteine; and mutant E for aspartic acid. To determine whether mutant A is defective in the isoleucine pathway, a combination of isoleucine plus valine was tested. See plate 21 in Figure 11. Explain the results.

Answer to Test Question

The mutant does not appear to be able to make aspartic acid from asparagine, but it may be able to make asparagine from aspartic acid. Therefore, aspartic acid could be the direct metabolic precursor of asparagine.

TEACHING TIPS

- Amino acid-based food additives or supplements include monosodium glutamate (MSG) and aspartame, a dipeptide ester of phenylalanine and aspartic acid.
- MSG may be used if students have identified a glutamate-deficient mutant.
- Aspartame may be used if students have identified either a phenylalanine- or aspartic acid-deficient mutant.
- Health food or nutritional supplements also might be a good source of specific amino acids.

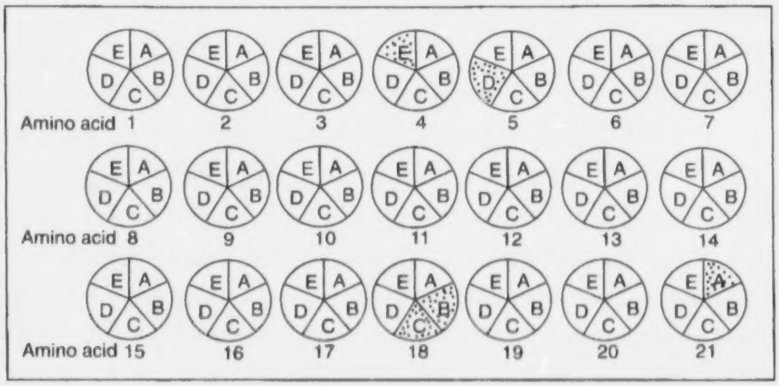

Figure 11. Each plate contains a different amino acid. See Table 3 to identify the amino acid by number. Each plate is streaked with nutritional mutants A, B, C, D, and E.

TEST QUESTION

A nutritional mutant selected from a bacterial library will grow on rich medium and on minimal medium supplemented with aspartic acid, but will not grow on minimal medium alone, or on minimal medium supplemented with a chemically similar amino acid, asparagine. What does the mutant suggest about the relationship between aspartic acid and asparagine?

VARIATION 22

The Effect of a Food Product on the Growth of a Nutritional Mutant of *E. coli*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 22.

SYNOPSIS

Students will use medium plates containing different amino acid-based food additives to identify which amino acid(s) are not synthesized by each mutant.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 4 minimal medium plates
- amino acid-base food additives or supplements.

SAFETY PROCEDURE

Do not ingest the food products.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Follow the directions for setting up Variations 1 to 21, but replace the amino acids in the growth medium with food additives known to contain the missing amino acids required by nutritional mutants.
- If autoclaving would denature the amino acid of interest in the food (see Table 2), make an extract or solution from the food and filter sterilize the extract.
- Prepare plates with a range of concentrations of the food product, as it will be difficult to determine the optimum concentration for bacterial growth beforehand.

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HYPOTHESIS GENERATION

Question

Can foods or foods supplemented with amino acids provide nutritional mutants with the needed amino acid for growth?

Sample Hypothesis

If a food product contains the amino acid that a nutritional mutant is unable to make, it will have the same effect on the growth of the mutant as will the amino acid alone.

Rationale

If a food product contains amino acids needed by a nutritional mutant, bacteria will grow on minimal media supplemented with these foods.

Sample Experimental Procedure

1. Have students identify the nutritional mutant they have found using the procedures for Variations 1 to 21.
2. Identify food products that contain the missing amino acid.
3. Have students bring to class foods that they hypothesize should provide the needed amino acid for the nutritional mutant.
4. Streak bacteria on the plates prepared by the teacher to determine whether the food product that contains that amino acid will have the same effect on bacterial growth as adding the amino acid.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Mutant	Amino acid required for growth on minimal medium	Growth on minimal medium + aspartame		Growth on minimal medium + monosodium glutamate (MSG)	
		1 mg/L	10 mg/L	1 mg/L	10 mg/L
A	Glutamic acid	-	-	-	+
B	Proline	-	-	-	-
C	Aspartic acid	+	+	-	-
D	Serine	-	-	-	-
E	Phenylalanine	+	-	-	-

Table 4. Five nutritional mutants grown on minimal medium supplemented with two concentrations of MSG and aspartame.

TEST QUESTION

You are given a "mystery peptide" composed of three different amino acids and a collection of bacterial nutritional mutants each deficient in a single amino acid. Design an experiment to determine the composition of the "mystery peptide."

Interpretation

Neither MSG nor aspartame contains proline or serine, so these supplements do not promote growth of nutritional mutants B and D. Mutant A will grow on MSG that supplies its missing amino acid, glutamic acid, but only at the higher of the two concentrations. Aspartame supplies aspartic acid to mutant C, which will grow at either concentration of the additive. Aspartame also supplies phenylalanine to mutant E, but too much of the additive appears to inhibit this mutant's growth.

Answer to Test Question

Apply a sterile solution of the "mystery peptide" to a minimal medium plate. Streak each of the nutritional mutants on the plate. Identify which three mutants grow on the supplemented minimal plate.

Isolation of Nutritional Mutants of *E. Coli*

Directions for Students

INTRODUCTION

Animals, plants, and even simple organisms, such as bacteria, contain thousands of different types of proteins. With very few exceptions, all proteins are built from a set of 20 amino acid "building blocks." In order to function properly, living things must have enough of each of these 20 amino acids to make all the proteins they require.

Your body obtains amino acids whenever you eat food that contains protein. That's the only way humans can obtain certain essential amino acids that are not produced by the body, including methionine, threonine, valine, and leucine. Your body can produce the other amino acids, known as nonessential amino acids, from chemicals obtained from other non-protein sources. It also can convert some types of amino acid into others.

Different organisms vary considerably in their ability to synthesize specific amino acids. Unlike humans, the bacterium *Escherichia coli* (*E. coli*) can produce all 20 amino acids beginning with ammonia and other simple chemicals. The ammonia is changed through a series of chemical reactions into the needed amino acid. Nature efficiently changes the ammonia in a series of sequential reactions called a metabolic pathway. Through metabolic pathways, energy is captured and used efficiently to produce the needed amino acid of the bacterium. If something interrupts one of these pathways, however, the bacterium cannot grow unless the formation of the missing amino acid is provided.

In this lab, you will use a collection of mutated *E. coli* known as a library. It was created by infecting bacteria with a bacterial virus that can insert randomly one piece of its own DNA into the bacterial genome. The rest of the viral DNA has been lost, because this virus is not able to survive or grow in this bacterial strain. If the invading piece of DNA, known as a transposon, inserts itself into a bacterial gene, it typically blocks that gene's function. See Figure 1. Each bacterial cell in a transposon-mutated library contains one transposon somewhere in its genome inserted into one of its genes.

When a transposon inserts into a gene required to produce a particular amino acid of a bacterial cell, the resulting cell is known as a nutritional mutant and cannot grow and divide to form a colony unless the missing amino acid is supplied. In this library, nutritional mutants occur at the rate of approximately 1 out of every 200 colonies tested. Usually, each nutritional mutant fails to produce only one amino acid. However, in the case of isoleucine and valine, which are produced by overlapping biochemical pathways, a single mutation can block both biochemical pathways and cause a requirement for both amino acids.

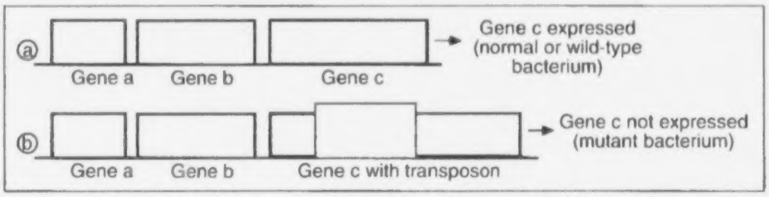

ⓐ
Gene a
Gene b
Gene c
Gene c expressed (normal or wild-type bacterium)
ⓑ
Gene a
Gene b
Gene c with transposon
Gene c not expressed (mutant bacterium)

Figure 1. Diagram showing the insertion of a transposon within a gene.

OBJECTIVES

At the end of this lab, you should be able to:

- Explain that bacterial species show genetic variations.
- Design an experiment to differentiate mutant from wild-type *E. coli* bacteria.
- Explain the relationship between individual bacteria and a colony.

SAFETY NOTES

Clean lab tables with 10% household bleach solution before and after use.

Wash hands thoroughly with warm water and antibacterial soap before and after lab.

Place all materials that have touched bacteria into a biohazard bag and autoclave. If an autoclave is not available, sterilize with 20% household bleach solution for one hour before disposal.

Exercise caution with clothing, hair, and other flammable materials while working near open flames.

Handle glass with care. Place broken glass in a designated container, not with general trash.

Always wear goggles and aprons.

Tie back long hair and secure bulky clothing.

Wear nonallergenic, disposable gloves when plating bacteria.

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of potential nutritional mutants. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment which gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. Why is controlling variables important?
3. What variables must be controlled in order to investigate this question? How might this be accomplished?

PLAN OF INVESTIGATION

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow. Make a numbered list of the steps you will use to investigate the topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. Are herbivores potentially at greater or lesser risk of suffering from the lack of essential amino acids? Why?
2. What are some evolutionary pressures that may have resulted in plants not containing or requiring very small amounts of certain nutrients in their tissues?
3. How can you identify a defective metabolic pathway of an organism?
4. If an organism has a defective pathway for a specific amino acid, could any other amino acid act as its substitute? Why or why not? How could you find out?
5. How might you use living organisms to determine the amino acid content of certain foods?
6. How might the amino acid content of a food be changed by the way it is prepared? How could you demonstrate this?
7. How could a transposon act to change the resistance of a bacterium to an antibiotic?

DESIGN OF VARIATIONS OF CORE EXPERIMENT

After collecting and analyzing the data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for experiments you could do next. Think of questions that occurred to you as you conducted the Core Experiment on nutritional mutants.

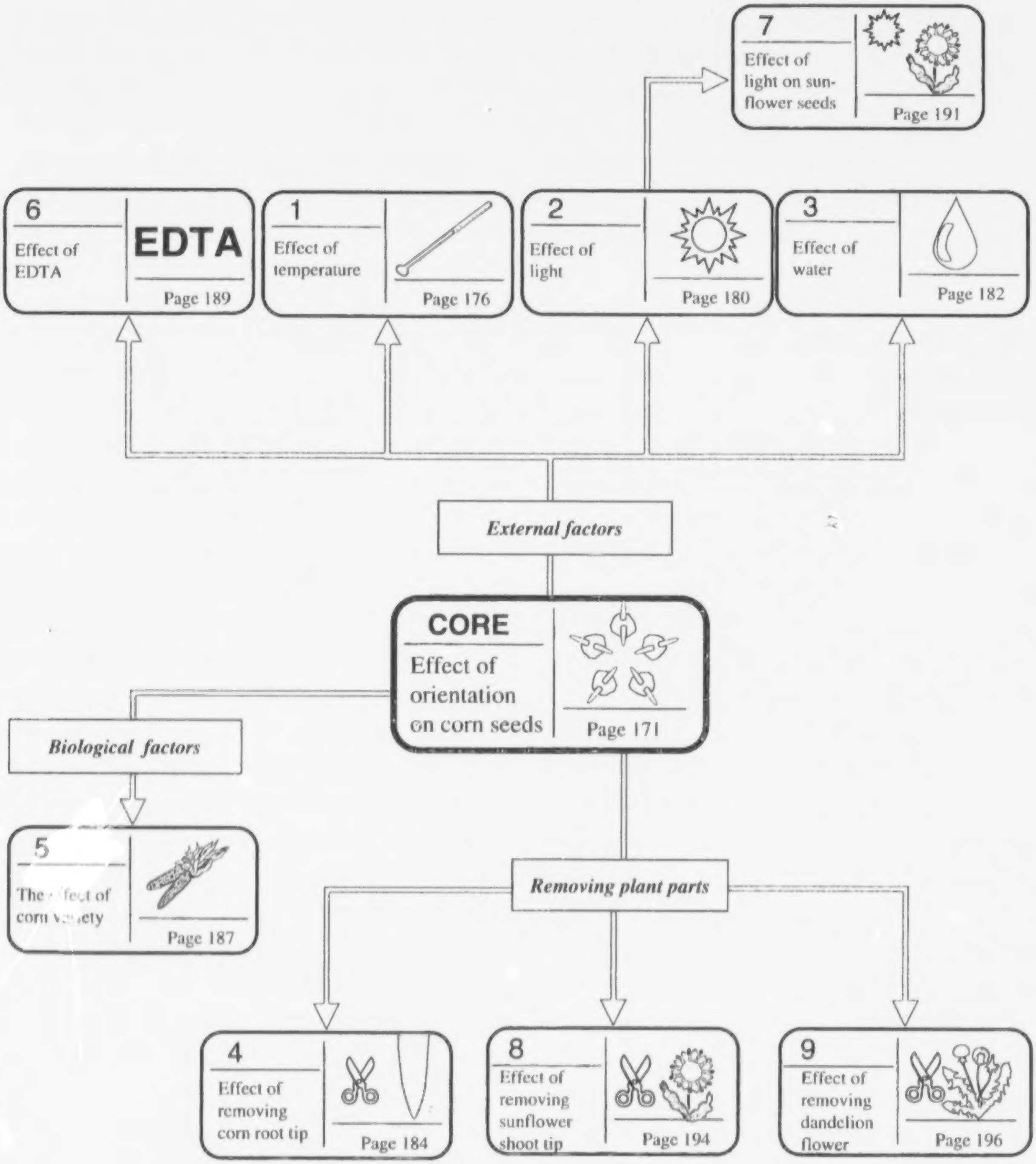
Design an experiment that is quantifiable and write your procedure in a numbered list of steps. Questions that other students have studied include the following:

- Can the amino acid necessary for growth of a nutrient mutant be identified?
- Can food or foods supplemented with amino acids provide nutritional mutants with the needed amino acid for growth?

NOTES

Which Way To Grow

At-A-Glance Map of the Core Experiment and Its Variations


7
Effect of light on sunflower seeds
Page 191
6
Effect of EDTA
EDTA
Page 189
1
Effect of temperature
Page 176
2
Effect of light
Page 180
3
Effect of water
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External factors
CORE
Effect of orientation on corn seeds
Page 171
Biological factors
5
The effect of corn variety
Page 187
Removing plant parts
4
Effect of removing corn root tip
Page 184
8
Effect of removing sunflower shoot tip
Page 194
9
Effect of removing dandelion flower
Page 196

NOTES

Which Way To Grow: Exploring Tropic Responses of Plants

SYNOPSIS FOR CORE EXPERIMENT

Students will assess the influence of seed orientation on the direction of root growth in corn seeds.

APPROPRIATE BIOLOGY LEVEL

Introductory or advanced

TEACHER PARTNERS

Anthony Umelo and Navarro Bharat
Anacostia Senior High School
16th and R Streets, SE
Washington, DC 20020

Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 176. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Explain how gravity is thought to cause the geotropic response in a corn plant.
- Measure angular differences in root growth and interpret these data with statistical analysis.

MATERIALS NEEDED

You will need the following for a class of 24:

- 1 shallow, 24 x 28-cm glass pan
- 1 L distilled water
- 144 corn seeds

You will need the following for each group of two students in a class of 24:

- 1 package of mounting putty or 12 straight pins
- 12 presoaked corn seeds
- 4 pieces of 10-cm filter paper or 10 x 10-cm chromatography paper
- 1 plastic food storage box with lid
- 1 protractor
- 1 pencil

SAFETY PROCEDURE

Handle fungicide-treated seeds with forceps or gloved hands. (See Teaching Tips.)

Use glues or sealants in a hood or ventilated area.

DIRECTIONS FOR SETTING UP THE LAB

One day before

Place seeds in a shallow pan of water 2.5 cm deep overnight for 12 hours.

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Observe sample root orientations, then discuss and form hypothesis.

Day 2 (20 minutes)

- Attach seeds to paper and arrange them in germination chamber.

Day 3 or 4 (45 minutes)

- Collect and share data. Introduce analysis to be completed outside of class or on another day when individual variations are designed.

PREPARATION TIME REQUIRED

10 minutes

- Gather supplies.

12 hours

- Soak the seeds.

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TEACHING TIPS

- Corn is not bred for a gravitropic response, so one may expect a large variation in response even in the same seed lot of a variety (Matthews & Zobel, 1995). Compensate by pooling data to create a large sample of 50 or more plants.
- The scientific name of corn is *Zea mays*.
- It is important to provide a smooth surface for the growth of roots so that they do not respond to obstacles, such as wrinkles in the paper or small moisture differences, rather than gravity.
- Presoaking the seeds makes it easy to pin them through the endosperm. Alternatively, you can use mounting putty or glue to affix them to the paper while they are dry, and then soak them overnight before orienting them in the germination boxes. Glues that work well include Amazing Goop™, Duro® Super Glue, RoSS™ Rubber Cement, and Dap™ Bathtub 100% Silicone Sealant. **Check the labels of these products for hazards and any safety considerations.** These adhesives hold tight when wet and do not inhibit germination when applied to dried seeds. Very sweet corn or "Supersweet" corn varieties may break the bond with an adhesive as the seed hydrates. Most seeds will hold fast.
- If you do not have equipment to project the results of a single sample for introducing the experiment, you may photocopy the germination chamber and make a transparency of it. You also can scan the results of a petri dish and display them on the computer monitor or print a transparency. Alternatively, you could pass the chamber(s) from student to student.
- Sweet corn seeds shrivel. It is difficult to identify or orient the embryo in some varieties. If you presoak the corn, it will plump and the embryo will be easier to see. See Figure 1.
- Sweet corn is frequently treated with fungicide. The fungicide will reduce problems with contamination, but requires that you take extra care in handling

(continued on p. 171)

TEACHER BACKGROUND

Content Information

Corn, like almost all plants, grows vertically with the shoot upward and the root downward. Plants, as germinating embryos, are responsive to up and down. If they were not, their shoots would not reach the light needed for photosynthesis, and their roots would not reach water and nutrients essential for growth and metabolism. The plant's response to up and down is called gravitropism (formerly called geotropism).

Plant signal perception is an evolutionary adaptation to the immobile nature of plants, giving them an ability to respond to environmental stimuli. Perception of gravity is thought to occur by sedimentation of amyloplasts in specialized cells called statocysts located in the root cap (Konings, 1995). The gravity stimulated curvature of a root is controlled by the movement of auxin that inhibits root growth. Lateral roots accumulate a higher concentration of auxin on their lower side. Elongation of cells on the lower side is inhibited relative to the cells on the upper side, and the root curves downward. High auxin concentrations stimulate stem growth. The connection between auxin concentration and amyloplast sedimentation is not entirely clear, but the sedimentation may somehow cause a movement of calcium ions that influence the transport of auxin.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

Correct Concept	Misconception
• A seed is composed of differentiated tissue. Only the embryo develops into a new plant.	• The entire seed grows into a plant.
• Cotyledons are immature leaves of the plant embryo. The cotyledons of dicots, such as bean, no longer contain endosperm but still serve as food reserves. In monocots such as corn, endosperm is still present but separate from the cotyledons.	• Both monocot and dicot cotyledons are immature leaves of the embryo that contain endosperm.
• Seeds may be germinated in vitro and embryos grown on chemically defined media.	• Seeds must be planted in soil in order to grow.
• Specific plant hormones promote differential growth.	• Fertilizers are the only chemicals that promote seed growth.
• Seeds are living.	• Seeds are nonliving.

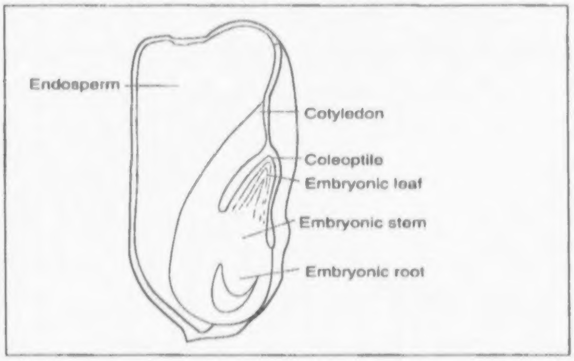


Figure 1. The structure of a corn seed.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Present five or six kinds of seeds that might be planted in gardens in your area. Tell a story about your father or mother always insisting that you plant seeds right side up. Ask students if you were just being teased about taking more time to plant those seeds, or if your parent was serious. Ask them if they can tell which is the right side on the seed. For most seeds this is very difficult. They are unlikely to be able to tell.

Four or five days before introducing the lab, affix the corn seeds on the filter paper with mounting putty in the pattern shown in Figure 2. Soak and germinate the seeds.

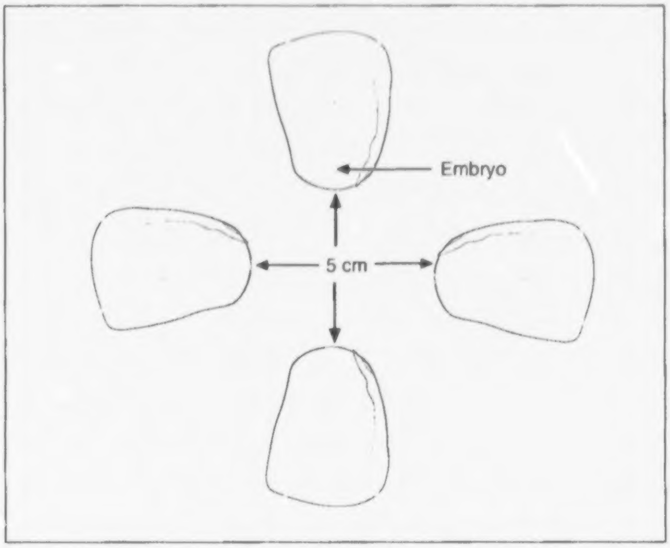


Figure 2. Seed positioning pattern for introducing the lab.

As the students make observations of the specimens, the teacher can prompt a discussion with the following questions:

- Can you tell which is right side up for these corn seeds?
- Would it make a difference how they were oriented when they were planted?
- In which direction is each root and shoot growing?
- Which way do you think was up when these plants started to grow?
- Discuss and demonstrate regular measurement techniques used in the Core Experiment.

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses. Questions that may be elicited include the following:

- What would happen to a seedling that did not grow the way these seedlings are growing?
- What could influence how a seedling grows?
- What is the cue to send the shoot toward the surface if a seed is planted deeper in the soil than light penetrates?
- What would happen if these seedlings were germinated in space?

TEACHING TIPS

(continued from p. 170)

the seeds. You can recognize the fungicide by its bright pink color.

- Do not reorient the seeds once the germination chamber has been set up. A root may respond by changing the direction of growth when stimulated by gravity for only 2 minutes (Gonick, 1997) or in as little as 27 seconds (Perbal & Ecole, 1995).
- Normally, embryo and root or shoot angles are measured from the vertical. Straight up is considered 0° and straight down is considered 180°.
- Do not incubate the seeds in total darkness. The gravity response is reported to be stronger in roots exposed to white light (Kelly & Leopold, 1992).

TEACHER'S NOTES

Sample Hypotheses

- If gravity is the cue that orients root growth, corn seeds planted at various angles will all have roots growing in the same direction.
- If a temperature differential is the cue that orients root growth, seeds heated from below and cooled from above will not produce roots oriented downward.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 175.

Question

Is there a difference in angle of root growth and the displacement of the embryo if corn seeds are oriented with the embryo tilted from its horizontal axis?

Hypothesis

If corn seeds are oriented with the embryo tilted from its horizontal axis, then the difference between angle of root growth and the displacement of the embryo from the vertical position will increase with reference to the angle of the force of gravity.

Rationale

Roots of germinating plants must anchor the plant and reach water and nutrients even if they are not hand planted by humans. A large difference between orientation and final position indicates a great orientation to gravity by the root.

Procedure

1. Remove 12 presoaked corn seeds from the pan of seeds soaked overnight. Place on a paper towel.
2. Draw 3 small dots with a pencil on each of 4 pieces of filter paper. Position the dots 0.5 cm from the top of the paper at 2.0, 4.5, and 7.0 cm from the left edge. See Figure 3.
3. Mount the presoaked corn seed on each dot of the paper as shown in Figure 3. Do not allow the seeds to dry while you work with them.

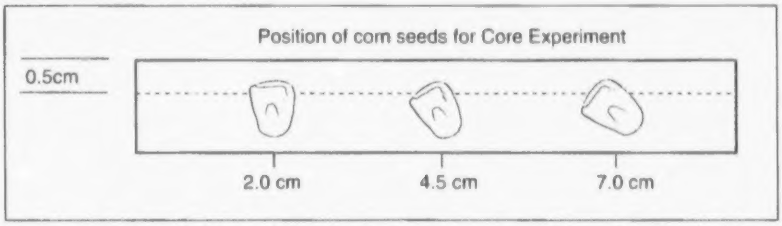


Figure 3. Position of corn seeds for the Core Experiment. Seeds may be affixed with mounting putty or straight pins.

4. Wet the paper completely.
5. Create a germination chamber by placing a little mound of modeling clay at 8 evenly spaced locations on the bottom of a plastic box.
6. Use the straight edge of your protractor to make a groove parallel to the far side of the box through each clay mound.

7. Stand each piece of filter paper in a pair of the grooves.
8. Add water to the bottom of the dish to 0.5-cm depth. Be certain that the entire bottom edge of each piece of paper is in the water.
9. Seal the lid on the germination chamber and incubate the seeds at room temperature.
10. When the root is 10 to 15 mm long, measure the angle of embryo displacement and the angle of root growth.
    a. Draw a straight line through the long axis of the embryo.
    b. Draw a straight line from the hypocotyl to the tip of the root.
    c. Use a protractor to measure the angles in degrees from the vertical line as indicated in Figure 4 and record your results in a table.

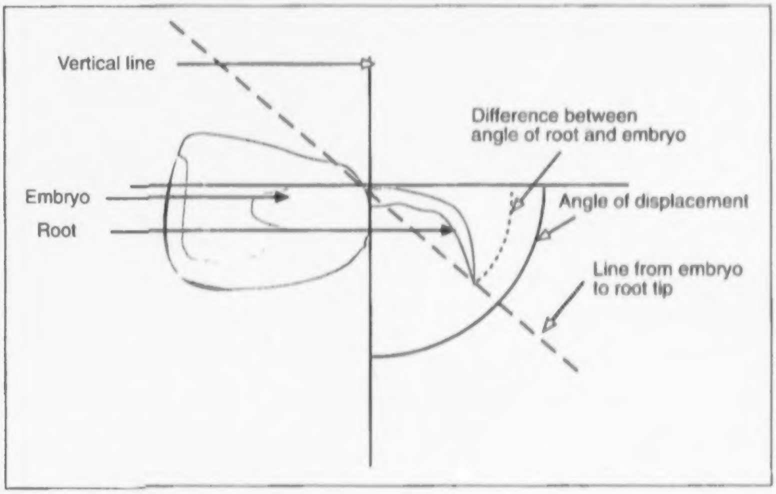

Vertical line
Difference between angle of root and embryo
Embryo
Angle of displacement
Root
Line from embryo to root tip

Figure 4. Angles to measure for determining root response to gravity.

11. Pool class data for analysis.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 1. Effect of corn embryo angle on the angle of root growth.

Student team	Angle of displacement	Difference between embryo and root growth angles
1-Plant 1	43	50
1-Plant 2	77	37
1-Plant 3	120	82
1-Plant 4	33	7
2-Plant 1	93	33
2-Plant 2	90	61
2-Plant 3	93	91
2-Plant 4	105	87

TEACHER'S NOTES

Interpretation

The data support the hypothesis that root response to gravity results in a stronger root curvature when the embryo is tilted more from the vertical.

Answer to Test Question

Answers may vary.

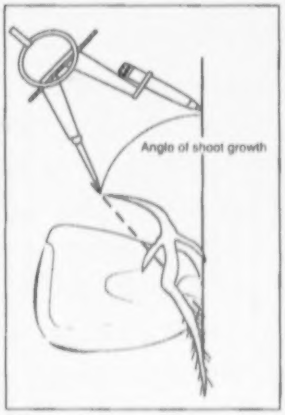
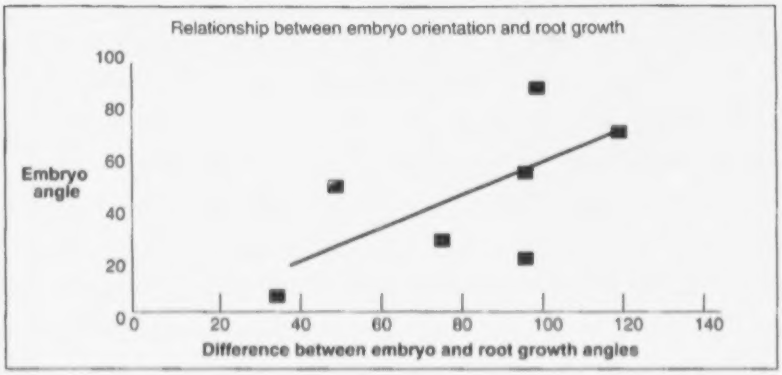


Figure 5. Instructions for measuring shoot angle of corn seedlings.

Draw a straight line from the tip of the shoot to the vertical line on which the seed was glued. Use a protractor to measure the angle between the line you have just drawn and the vertical line.



Graph A. Comparison of corn embryo orientation and final root growth angle after 3 days incubation in the dark at room temperature. Angles were measured in degrees. The line is a least squares regression line.

TEST QUESTION

Suppose you are interested in testing the gravitropic response of shoots. How would you measure that response? Draw a figure and write an explanation to clarify your technique.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted their first experiments. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- What other environmental factors might influence gravitropic responses? How might you independently measure the effects of these factors? Why do these factors influence gravitropic responses?
- What other environmental stimuli might plants respond to and why? How would you test these responses independent of one another?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

Materials that are needed by students who are visually impaired include a braille protractor, braille ruler, braille paper, braille graph paper, stylus, wax pencil, rubber mat or base for making raised-line drawings, tracing wheel, blunt tip tools for drawings, talking thermometer, talking watch or timer, and rubber gloves for handling fungicide-treated seeds.

- Provide the blind student with a copy of the lab experiment in braille with graphs on braille graph paper and raised-line drawings. Plant experiments often can be performed by students who are blind.
- Provide interpretation of the direction "mount seed as shown in Figure 3."
- Explain where the *hypocotyl* is located in Step 10. Locate supplies within easy access of students who are blind to allow them to do this step unaided.

Deaf or Hard-of-Hearing

No special modifications are needed for this experiment.

Gifted

- Have gifted students design experiments to propose possible explanations for the gravitropic responses on the cellular level. Challenge them with questions such as, "Are gravitropic responses due to changes in the distribution of cells or hormones within plant tissues?"
- Encourage students to undertake studies to determine what part environmental factors and/or genetic factors play in the plants' responses to gravitropism. A combination of library research and discussions with scientists in nearby universities or research facilities will help in this regard.

Mobility Impaired

The general directions for performing this activity can be followed by students in wheelchairs. See "Allelopathy" for details.

- Have manually impaired students act as observers. This experiment requires fine motor skills to handle the seeds and seedlings.
- Provide access to a computer if manually impaired students have computer skills to compile data and do graphing.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. If an embryo is at an angle of 90° and the root grew downward at 60°, how far from the vertical position would the root be growing? How does this root's orientation to the vertical compare with your observations?
2. Why was it desirable to pool class data to evaluate the effect of gravity on the angle of root growth?

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TEACHER'S NOTES

Answers to Additional Test Questions

1. This root would be only 30° from straight down. Our average angle between the root and the vertical line is about 24°, so these values are similar.
2. Corn plants vary widely in their response to gravity. To detect a pattern, it is necessary to have a large sample size.

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VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: *Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.*

VARIATION 1

The Effect of Temperature on the Gravitropic Response of Corn

SYNOPSIS

Students will compare the root angle of the same variety of corn under two or more temperatures.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24 students:

- Two or more temperature controlled environments, such as incubators or water baths.

HYPOTHESIS GENERATION

Question

What is the effect of increased temperature on the root growth angle of corn seedlings?

Answers to Questions and Analysis on Student Page

1. The graph should be similar to Graph A. The combined class data show that the greater the angle of the embryo, the more the root curved.
2. These data support the hypothesis. The roots do compensate for greater original disorientation by growing with a greater curvature, finally growing nearly straight down.
3. If one wanted to create a variety of corn with as great a tropic response as the most strongly responding plant, one would clone it. Alternatively, one could self-pollinate the mature plant, or at least cross pollinate two plants with strong responses. Cloning would be faster and probably less expensive.
4. Other stimuli that could cause the roots to change their angle of growth might be touch, such as that presented by coarse grains in the soil, and a directional supply of water.

TEACHING TIPS

- Most classrooms are not controlled at the same temperature throughout the day. You could use an unheated basement, where the temperature is fairly constant over a period of several days, and a deep pan of water with an inexpensive aquarium heater to provide two temperature regimes if you do not have incubators or water baths.
- Matthews and Zobel (1995) found variable responses of corn roots to different temperatures. The roots grew most directly downward at 25°C.

Sample Hypothesis

If the temperature is increased, then the root growth angle of corn seedlings will increase.

Rationale

It would be adaptive for roots to grow downward more directly if it is hot, because high temperatures are often accompanied by drying conditions.

Sample Experimental Procedure

Repeat the Core Experiment placing some germination chambers in each of two or more constant temperature environments.

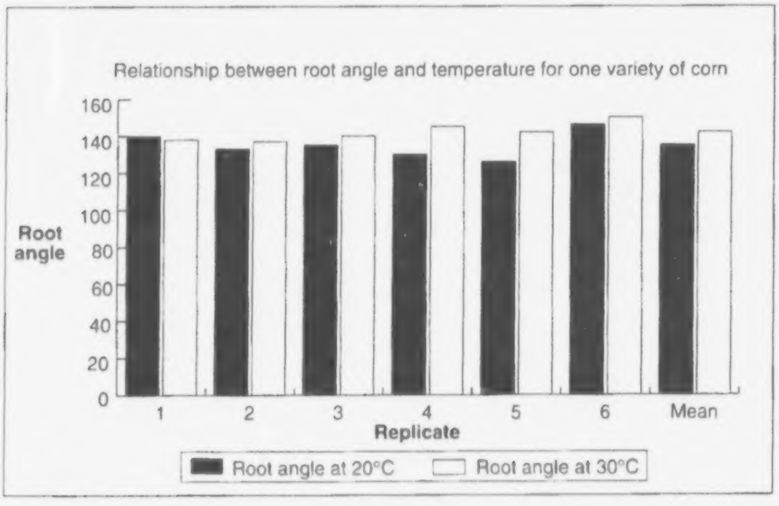
TEACHER'S NOTES

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 2. Corn root growth angles measured after incubation in 2 different constant temperature environments.

Replicate	Root angle at 20°C	Root angle at 30°C
1	140	138
2	133	137
3	135	140
4	130	145
5	126	142
6	146	150
Mean	135	142



Graph B. Root angle and temperature relationship.

TEACHER'S NOTES

An appropriate analysis for data collected in this design would be a Wilcoxon Two-Sample Test (Sokal & Rohlf, 1995). To perform the statistical test:

1. Convert measurements to ordered ranks and sum the ranks:
   a. Keep track of 20°C and 30°C values as you order them together from low to high. It is often easiest to do this if you use different colors or two columns to identify these data sets.
   b. Assign ranks to the ordered values. If two observations have the same value, assign them the average of their ranks. For example, both sets of data have the value 140. The ranks for this value are 7 and 8, so each value is assigned the average of (7 + 8) or 7.5.
   c. Sum the ranks of the smaller sample if the samples are of unequal size. Call this smaller sample n_2, and let n_2 be the number of observations in that sample. The sum of the ranks of the small sample is $\sum R_2$. Call the other sample n_1, and let n_1 be the number of observations in that sample. The sum of the ranks of that sample is $\sum R_1$. Sum the ranks of either sample if they are of the same size.
   d. Table 3 shows these steps produce:

Table 3. Result of Wilcoxon Two-Sample Test.

	20° Root angle		**30° Root angle**	
	Ordered value	**Rank**	**Ordered value**	**Rank**
	126	1		
	130	2		
	133	3		
	135	4		
			137	5
			138	6
	140	7.5	140	7.5
			142	9
			145	10
	146	11		
			150	12
Sum of the ranks ($\sum$R)		28.5		49.5

2. Compute: Compute the Wilcoxon statistic by substituting your values in the formula
   $C = n_1 n_2 + [n_2(n_1 + 1)/2] - \sum R_2$
   Also calculate C by the formula
   $C' = n_1 n_2 - C$
   Here these computations produce
   $C = 6(6) + [6(6 + 1)/2] - 28.5 = 28.5$
   and $C' = 6(6) - 28.5 = 7.5$
3. Compare: Use the larger of the two values, C or C', as the test value. Compare your value with the tabled value.

Table 4. Critical values at 5% for Wilcoxon Two-Sample Test.

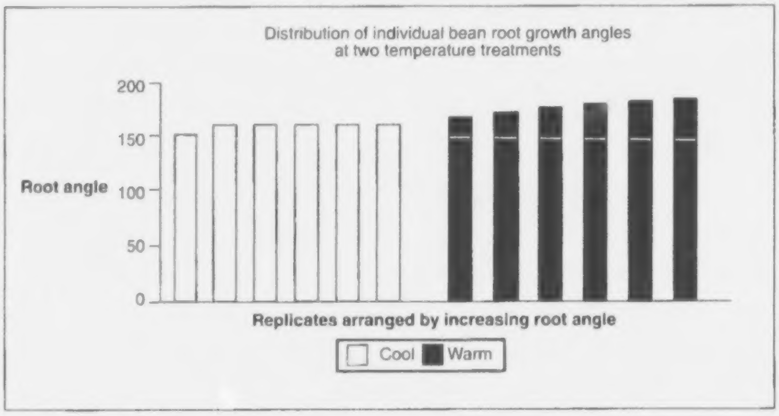
n_1	n_2	**Comparison value**
3	3	9
4	3	12
4	4	15
5	2	10
5	3	14
5	4	18
5	5	21
6	2	12
6	3	16
6	4	21
6	5	25
6	6	29

These steps produce:
$C_{calculated} < C_{tabled}$ or $28.5 < 29$

4. Conclude: If your value is less than the tabled value, the apparent differences are most likely the result of chance alone and do not represent significant differences in these data sets. If your value is equal to or greater than the tabled value, the differences in your data sets are not due to chance alone.

TEST QUESTION

The graph below displays data collected by another group of students studying the gravitropic response of a single variety of beans to two different temperatures. Does temperature alter the response of the roots of this variety of beans? Is it necessary to analyze these results statistically? Explain.



Graph C. Influence of two constant temperature regimes on the gravitropic response of bean roots.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide a talking thermometer for the student who is blind.
- Consider that it may be more difficult for some students to read columns of figures and graphs with small variations by touch than for others to read them by

Interpretation

Statistical analysis reveals that the responses of the roots to gravity do not differ at the two temperatures tested. The variation within a population means that one should probably use a larger sample. However, at this point it must be concluded that the hypothesis is not supported by these data.

Answer to Test Question

These students found that the bean roots grew more vertically at the warm temperature. It is not necessary to analyze these data statistically because, although the curvature of all the roots is similar, the cool temperature curvature is always less than the warm temperature curvature and the variation within the samples is similar.

sight. A wax pencil can be used for one of the bar graphs. A tracing wheel or other tool can be used for the second bar.

VARIATION 2

The Effect of Light on the Gravitropic Response of Corn Roots

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will compare the angle of root growth of corn seedlings after changing their orientation and exposing them to light or keeping them in the dark.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- 12 corn seedlings with straight roots about 1 cm long
- 1 light source
- 2 dark cabinets or boxes
- 1 heat trap
- 1 clear, colorless germination container

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Use the setup in Steps 1 to 8 of the Core Experiment to germinate corn seedlings. Do not vary the angle of the seed. Adjust the seeds so that the roots emerge directly down.

HYPOTHESIS GENERATION

Question

What effect will light from below have on the gravitropic response of corn?

Sample Hypothesis

If corn roots are lighted from below, the apparent gravitropic response will be less than if the roots are placed in the dark.

Rationale

Roots normally grow in the dark. They may have a negative phototropic response that appears as a stronger gravitational response. Light from below could send the wrong signal.

Sample Experimental Procedure

1. Divide the roots into matched control and treatment groups.
2. Set up the control group.
   a. Reorient the roots to the perpendicular either by cutting the paper that they have germinated on or by rotating them.
   b. Make a mark on the paper just at the tip of the root.
   c. Return the seedlings to their germination chamber.
   d. Shield the chamber from light by putting it in a dark cabinet or box.
3. Set up the treatment group.
   a. Reorient the roots to a perpendicular position by cutting the paper on which they have germinated and rotating it.

TEACHER'S NOTES

TEACHING TIPS

- Clip-on shop lights are handy light sources that can be used to vary the intensity of the light by changing light bulbs.
- Clear, colorless baking pans make good heat traps when filled with water. They can be supported on two narrow boards over the light source.
- A glass bread pan covered with plastic wrap makes a good clear, colorless germination chamber.
- Roots kept in the dark have a far smaller (10°) gravitropic curvature in 2 hours than do roots exposed to white light (60°) (Kelly & Leopold, 1992). The response of root gravitropism is consistent with light-stimulated biosynthesis.

b. Make a mark on the paper just at the tip of the root.
c. Place seedlings in a clear, colorless, covered germination chamber.
d. Shield the chamber from stray light by putting aluminum foil around the top and sides.
e. Place the chamber immediately over the heat trap.
f. Place the light immediately under the heat trap and turn it on.

4. After 2 hours, draw a dot at the position of each root tip or end and draw a line between the 2 dots marking the initial and end positions.
5. Measure and record the angle between a horizontal line drawn through the starting position of the root and the line you drew from that point to the end point. Record values above the horizontal as negative and those below the horizontal as positive.
6. Analyze the results of your measurements.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Angle of roots reoriented to a horizontal position and incubated with lights placed below or in room light for 2 hours. Measurements are in degrees from the horizontal. Growth below the horizontal position is measured as a positive number. Growth above the horizontal position is measured as a negative number.

Replicate	Root angle (°)	
	Room light incubation	Directional light incubation
1	60	90
2	60	70
3	60	50
4	34	58
5	65	28
6	60	42

Appropriate analysis is the Wilcoxon Two-Sample Test. Here C = 17 and C′= 27. The critical comparison value is 29.

TEST QUESTION

Some plants require light to germinate. Roots of these plants do not necessarily exhibit negative phototropic responses. What might be the adaptive value of a light requirement for germination?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Students who are visually impaired should have no difficulty with this experiment if they understand the Wilcoxon Two-Sample Test. Some math departments might assist with this.

TEACHER'S NOTES

Interpretation

These results do not support the hypothesis. The Wilcoxon statistic is less than the tabled critical comparison value, so one can conclude that differences as great as those observed in these data sets are likely to occur by chance alone.

Answer to Test Question

Roots and shoots have only the energy stored in the seed before the shoots reach light and begin to photosynthesize. If a seed can detect light it is likely that the shoot could reach light before running out of energy reserves. If this idea has any merit, one would expect to find a light requirement for germination more common among small seeded species than among large seeded species.

VARIATION 3

The Effect of Water on the Gravitropic Response of Corn Roots

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will determine if water will alter the gravitropic response of corn roots.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- 1 5 x 5 x 2-cm piece of absorbent cotton
- 1 dropping pipette
- 1 transparency film
- 12 corn seedlings with straight roots about 1 cm long

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Germinate seeds as in Core Experiment. Do not vary the angle of the seed. Place seed so roots emerge going down.

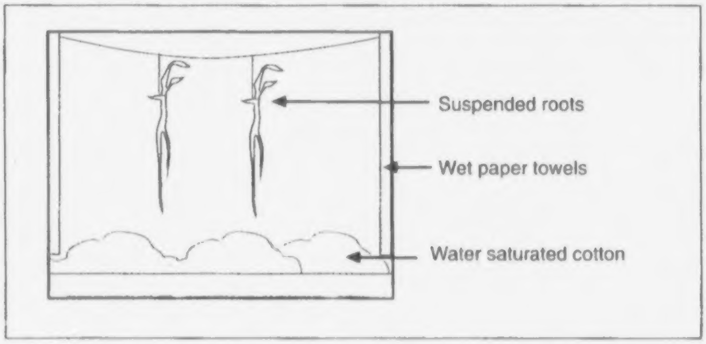


Figure 6. Cross section of a chamber to detect the interaction of hydrotropism and gravity on roots.

HYPOTHESIS GENERATION

Question

How will corn roots respond to gravity if the water source is not below the roots?

Sample Hypothesis

If corn roots are exposed to water that can be reached easily, but that is not located below them, they will not respond as strongly to gravity.

Rationale

A tropic response to water could be a mechanism for avoiding drought.

Sample Experimental Procedure

1. Divide the roots into 2 matched groups. One group will be the control and the other the treatment group.
2. Set up the control by repeating the following steps for each replicate.
   a. Fasten (glue or staple) a 5 x 5 x 2-mm piece of absorbent cotton at the right edge, about 1 cm from the upper edge of a piece of transparency film.

TEACHER'S NOTES

TEACHING TIPS

- Research in the 1800's showed that at least some plants grow toward regions of higher moisture. For some roots, hydrotropism can overcome gravitropism (Takahashi, 1994).
- Another possible design would be to line a box with wet paper towels to establish a uniformly humid chamber. In the middle of the chamber, suspend a pair of roots in the arrangement shown in Figure 6.

b. Affix a germinated corn seedling to the transparency film with the root horizontal and 1 mm from the left edge of the cotton. See Figure 7.

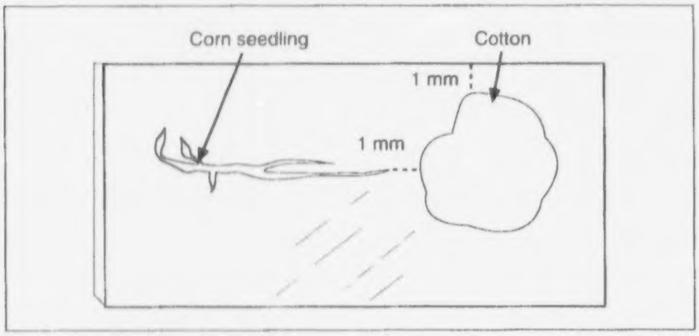


Figure 7. Seedling attachment to transparent film.

c. Make no additions to the cotton.
d. Return the plant to the germination chamber.
3. Set up the treatment by repeating Steps 2a and b for each replicate.
a. Saturate the cotton with distilled water.
b. Return the plant to the germination chamber.
4. After 4 hours, measure the angle of curvature.
a. Mark the position of the root tip.
b. Draw a line from that final mark to the initial tip placement.
c. Measure and record the angle between a horizontal line through the initial tip placement and the final mark.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Angle of root growth with and without a horizontally supplied water source.

Replicate	Root angle (°)	
	Dry cotton at root tip	Water saturated cotton at root tip
1	60	63
2	60	60
3	60	60
4	65	72
5	55	60
6	60	78

Use the Wilcoxon Two-Sample Test to analyze these data. Here C = 4 and C′= 32. The critical tabled comparison value is 29.

TEST QUESTION

Study the directions for performing the Wilcoxon Two-Sample Test for analyzing data like that collected in this experiment. How many samples are necessary to conclude that the differences observed are significant if there is no overlap in these data? Assume that you are content to make an error in your interpretation 5% of the time. The .05 column is all that you have been given. Explain.

TEACHER'S NOTES

Interpretation

These results do not support the hypothesis. Data sets as different as those found most likely differ by chance alone. One would expect less of a downward growth when water was supplied laterally, but the greatest downward curvature occurred then.

Answer to Test Question

Three samples are adequate if there is no overlap. The Wilcoxon statistic calculated will be

C = 3(3) + [3(4)/2] - 6 = 9 and
C′= 3(3) - 9 = 0.

The tabled comparison value is 9. Since the calculated value is equal to the tabled value, the differences in these data sets are not due to chance alone.

TEACHER'S NOTES

VARIATION 4

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide a lab partner if the impaired student needs assistance handling the roots for this investigation. The use of a dropping pipette to saturate the cotton with water presents no difficulty as long as the number of drops of water do not need to be counted.
- Provide a raised-line drawing of Figure 7, "Seedling attachment to transparent film" for the student who is blind.

VARIATION 4

The Effect of the Root Tip on the Gravitropic Response of Corn Roots

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS

Students will compare the gravitropic response of corn seedling tap root with and without 1 mm of the root tip removed.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 1 single-edge razor blade or scalpel
- 1 metric ruler
- straight pins, modeling clay, or glue
- 1 permanent marker or pencil

SAFETY PROCEDURE

If you use razors, use only those with single edges. Dispose of razors and scalpel blades in a sharps container.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Prepare corn seedlings as in the Core Experiment, but do not orient the seed other than with the tip of the seed pointing downward.

HYPOTHESIS GENERATION

Question

How is the gravitropic response of corn affected by the removal of its tip?

Sample Hypothesis

If the tip of a corn root is removed, then the gravitropic response of a corn root will be reduced.

Rationale

Roots grow from near the tip. The sensors for moisture, obstacles, and gravity must be near there to permit the root to change direction.

Sample Experimental Procedure

1. Germinate corn seeds as described in Steps 1 through 7 of the Core Experiment.
2. When the roots are about 1 to 1.5 cm long, divide the seedlings into 2 closely matched groups. Discard seedlings with crooked roots.

3. One group is the control.
   a. Carefully remove the seedlings from their growing situation.
   b. Blot each seed dry and fasten it to a fresh piece of paper so the root is perpendicular to its previous position.
   c. With a pencil or permanent marker, mark the position of each root tip on the paper.
   d. Completely wet the paper and place it in a germination chamber like the original.
4. The second group is the treatment group.
   a. Carefully remove the seedlings from their growing situation.
   b. Use a sharp razor blade to cut off the root 1 mm from the tip. See Figure 8.

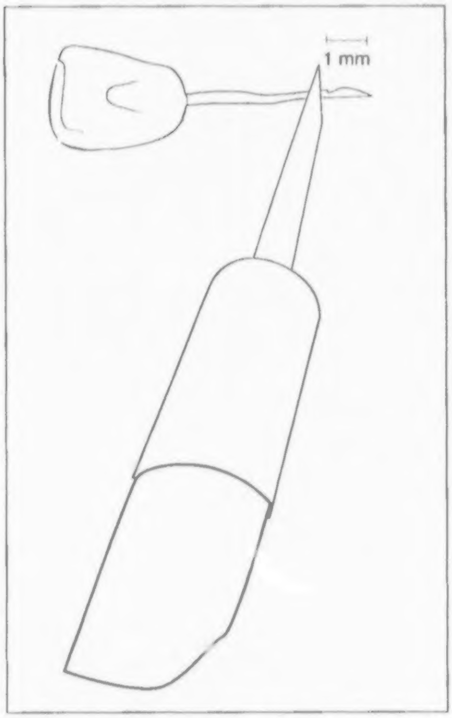


Figure 8. Removal of 1 mm of root tip.

   c. Blot each seed dry and fasten it to the paper or transparency film so the root is perpendicular to its previous position. See Figure 9.

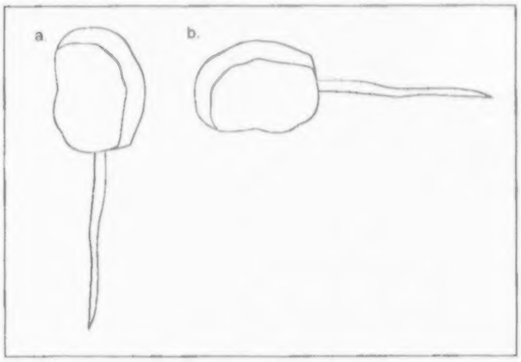


Figure 9a. Original orientation. **b.** Perpendicular orientation.

TEACHING TIPS

- Interested students may want to try removing less than 1 mm or more than 1 mm as additional treatments. This would be a good time to have them study the microscopic structure of roots in prepared root sections.
- Roots can be prepared by one class and analyzed by another. Or students can briefly mark and label the positions of their roots for measurement another day.
- Failure of the root to grow toward gravity does not conclusively demonstrate that the sensor is in the root tip. It could be that removing the tip stops the root from growing. To test this idea, allow the roots to incubate for 10 hours following the root tip removal. Then measure the growth.

TEACHER'S NOTES

Interpretation

The hypothesis is supported by these data. Intact roots had an average positive gravitropic response of 38° more than roots from which 1 mm was removed.

Answer to Test Question

See Graph D.

d. With a pencil or permanent marker, mark the position of each root end on the paper.

e. Place the seedlings in the germination chamber with the root horizontal. Completely wet the paper before placing the seedlings in the germination chamber.

5. After 4 hours, draw a dot at the position of each root tip or end and draw a line between the two dots marking the initial and end positions.
6. Measure and record the angle between a horizontal line and the line you drew. Record values above the horizontal as negative and those below the horizontal as positive.
7. Analyze the results of your measurements.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 7. Effect of removing 1 mm of root tip on the gravitropic response of corn seedlings over 4 hours.

Replicate	Root angle (degrees from horizontal)	
	Treatment (tip removed)	Control (root intact)
1	-10	30
2	-5	38
3	0	40
4	0	35
5	-5	35
6	0	30
Mean	-3	35

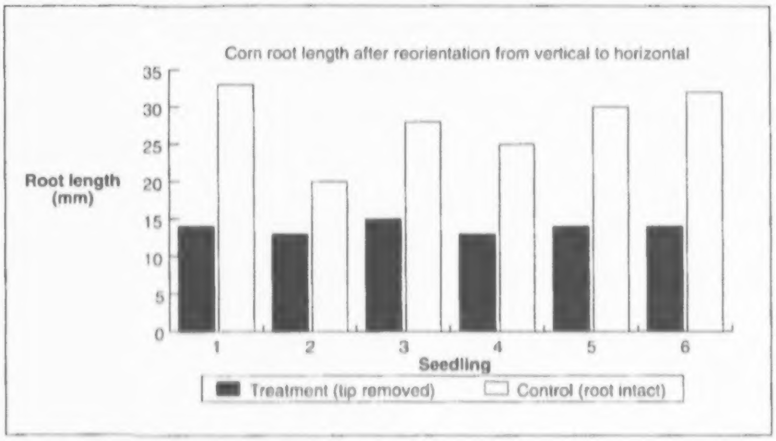
Draw a picture of two seedlings representing the average final root angle. Label the angle with the average degree from horizontal. Appropriate statistical analysis here is the same as in the Core Experiment.

TEST QUESTION

Another group of students measured the growth of roots with and without the terminal 1 mm removed. In every other way, they handled the roots as done in this experiment. Graph their results presented in Table 8 and write a conclusion.

Table 8. Root length 10 hours after roots were reoriented perpendicular to gravity.

Seedling	Root length (mm)	
	Treatment (tip removed)	Control (root intact)
1	14	33
2	13	20
3	15	28
4	13	25
5	14	30
6	14	32



Graph D. Corn root lengths were measured 10 hours after they were reoriented from vertical to horizontal. One mm of the tip was removed on half the plants.

TEACHER'S NOTES

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- At the beginning of the course, discuss the use of razors and scalpels by students who are blind. Those who have used paring knives in the kitchen and those who shave with non-electric razors should have no difficulties.
- Mark positions of roots on the paper with a braille stylus.

VARIATION 5

The Effect of Corn Variety on the Response to Gravity

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Students will compare the angle of root growth for different varieties of corn.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- Several seeds of a second variety of corn.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Put the seeds of different varieties in water to presoak for 12 hours before affixing them on construction paper.

SAFETY PROCEDURES

Handle fungicide-treated seeds with forceps or wear gloves while handling them.

TEACHING TIPS

- Place the seeds of different varieties in the same germination chambers since conditions may vary slightly between chambers.
- If your students are confident that they can orient the corn seedling with the embryo in a horizontal position, they may want to use the design of the Core Experiment.
- Primary roots of corn will show positive gravitropism within 10 to 15 minutes (Lee, Mulkey, & Evans, 1983), so the time schedule for this exercise is flexible. However, measure the root angle made during the first 0.5 to 1.0 cm of growth after reorientation.
- You can save several steps if the roots are straight down. Simply cut the paper on which they have germinated and rotate the piece with the seedling 90°.

HYPOTHESIS GENERATION

Question

Would failure to detect a difference in gravitropic response between different corn varieties have provided conclusive evidence that the response is not under genetic control?

Hypothesis

If the root angle of corn varieties differs, then the gravitropic response of corn roots is regulated by genes.

Rationale

Although corn varieties are not selected for gravitropic responses, that trait is likely linked to a trait for which the corn is bred.

Sample Experimental Procedure

1. Draw a line 0.5 cm from the top long edge of the construction paper.
2. Affix seedlings of 2 different varieties of corn on construction paper and germinate them as in the Core Experiment. Be certain, however, to set the embryo vertically.
3. When the roots are 0.5 to 1.0 cm long, select those with straight, downward pointing roots.
4. Work quickly as you handle these seedlings so that the root hairs do not dry. Reorient these roots to the horizontal. They should be parallel to the original line you drew on the paper. Place a mark on the line at the tip of the root.
5. After 2 hours incubation in this new position, mark the position of the root tip on the transparency with a permanent marker.
6. Draw a line between the tip immediately after reorientation and the tip after further incubation. Measure and record the angle between the horizontal and the line you drew.
7. Compare the root growth angle for the 2 varieties.

DATA ANALYSIS AND INTERPRETATION

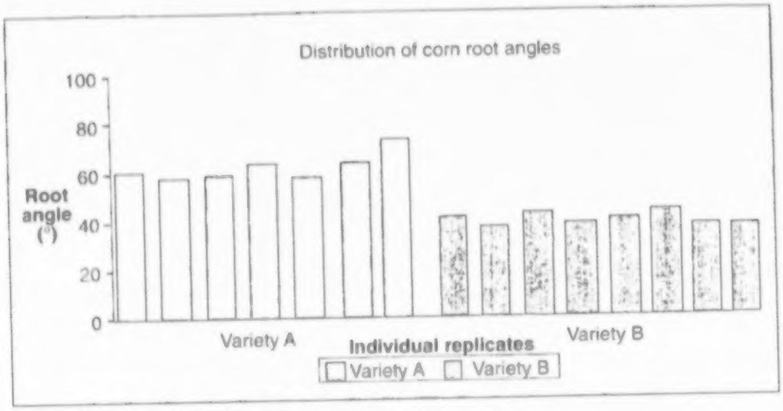
Sample Data

Table 9. Effect of gravity on root growth of two varieties of corn.

Replicate	Root angle (°)	
	Variety A	Variety B
1	60	39
2	58	37
3	59	42
4	61	38
5	63	40
6	58	42
7	63	37
8	70	37

Interpretation

The mean root angle for Variety A is 62°. The mean root angle for Variety B is 39°. These data show a strong difference in root growth angle response between Variety A and Variety B. Variety A has a more positive gravitropic response by an average of 22°. The gravitropic response of these corn varieties appears to be controlled by genes.



Graph E. Effects of gravity on two corn varieties.

TEST QUESTION

Would failure to detect a difference in gravitropic response between varieties have provided conclusive evidence that the response is not under genetic control?

VARIATION 6

The Effect of EDTA on the Gravitropic Response of Corn Roots

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will determine if adding a chelator of calcium (EDTA) will influence the gravitropic response of corn roots.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- absorbent cotton
- 2 dropping pipettes
- 2 mL of 50 mM EDTA
- transparency film
- 12 corn seedlings with straight roots about 1-cm long

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Germinate seeds as in the Core Experiment.
- To prepare 50 mM EDTA: Place 1.46 g EDTA in 80 mL distilled water. Then add NaOH pellets one at a time to dissolve the EDTA. Dilute to a final volume of 100 mL with distilled water with a pH near 8. Alternatively, use the dihydrate disodium salt of EDTA, which is more soluble. Add 1.86 g to 80 mL distilled water. If necessary, add NaOH pellets to dissolve the EDTA. Adjust the final volume to 100 mL with distilled water.

HYPOTHESIS GENERATION

Question

How will the addition of EDTA solution affect the corn root's response to gravity?

Answer to Test Question

If we had found no difference in the gravitropic response we could not conclude that the response is not under genetic control. It is possible for two populations to have the same genetic information for some traits. It is also possible that our techniques did not allow us to detect a difference that exists.

TEACHING TIPS

- Gradients of extracellular calcium are generated across the root tip of corn seedlings and are required for the gravitropic curvature (Bjorkman & Cleland, 1991).
- EDTA (Ethylenediaminetetraacetic Acid) chelates calcium and its effects on the gravitropic response of corn roots can be reversed by supplying calcium (Lee, Mulkey & Evans, 1983).
- Cotton cosmetic pads are excellent material to use here because they make the dimensions easy to control.
- Additional information needed for the test question is given with Variation 2.

Sample Hypothesis

If corn roots are exposed to EDTA, then they will not respond to gravity.

Rationale

Calcium has been shown to be associated with corn root gravitropism (Bjorkman & Cleland, 1991).

Sample Experimental Procedure

Repeat Steps 1 through 4 of Variation 3 with the following changes:
Step 2c. Saturate the cotton with distilled water.
Step 3a. Saturate the cotton with 50 mM EDTA.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 10. Root curvature exposed to EDTA or water.

Replicate	Angle of curvature (°)	
	Water (control)	EDTA (treatment)
1	60	0
2	60	0
3	60	0
4	68	0
5	50	0
6	50	0

An appropriate analysis for data collected in this design would be a Wilcoxon Two-Sample Test (Sokal & Rohlf, 1995). These values are so distinctly different with no overlap that no statistical analysis is necessary. No graphical display is needed.

Interpretation

EDTA prevented a gravitropic response. The hypothesis is supported by these data.

TEST QUESTION

A group of students was uncertain whether EDTA would chelate a gravitropically important ion other than Ca^{+2}. They decided to test whether 30 mM CaCl$_2$ would reverse the effect of EDTA. If calcium was the only gravitropically important ion chelated by EDTA, what should they find? How could they determine whether the Ca^{+2} or the Cl^{-1} was the restorative element?

Answer to Test Question

It could be expected that the CaCl$_2$ would restore the ability of the root to respond to gravity and that the roots would turn downward. They could rule out Cl^{-1} as the restorative element by testing with MgCl$_2$ (or MnCl$_2$) instead of CaCl$_2$. If this compound failed to restore the gravitropic response, then Cl^{-1} was not the restorative agent.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Remind students, especially blind students, to wear rubber gloves when handling seeds treated with fungicide.
- Refer to previous variations for other techniques needed for this variation.

VARIATION 7

VARIATION 7

The Effect of Light on the Apparent Gravitropic Response of Sunflowers

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students will determine whether light can overcome the gravitropic response of sunflower shoots.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 10 straight leafy shoots of 10-day-old sunflower seedlings
- 10 100-mL graduated cylinders
- Parafilm™
- light source
- 2 dark cabinets or boxes
- heat trap

SAFETY PROCEDURE

Make sure the heat-trap mounts are very stable so there is no danger of spilling water into the light fixture.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

1. Soak sunflower seeds and germinate in petri dish for 10 days.
2. Cut leafy shoots early in the morning. If necessary, remove leaves on some so that all shoots are approximately the same.

HYPOTHESIS GENERATION

Question

Is the gravitropic response of sunflowers stronger than its phototropic response?

Sample Hypothesis

If the gravitropic response of sunflowers is stronger than the phototropic response, then shoots will grow upward whether they are in dark or light.

Rationale

Day or night, sunflower shoots grow upward. Gravity is a constant force in the life of a plant, but light is a variable force. Major orientation should be responses to reliable cues. The leaves and flowers track the sun, so it is possible that some of the upward response results from phototropism.

Sample Experimental Procedure

1. Divide the shoots into 2 matched groups.
2. Completely fill the graduated cylinders with water. Use paper towels or cotton to anchor the stems in place with the shoots against the side of the cylinder.
3. Gently seal the shoots in the top with Parafilm™. See Figure 10.

TEACHING TIPS

- Clip-on shop lights are handy light sources in which you can vary the intensity of the light by changing light bulbs. You also can vary the light intensity by changing the distance between the light and the shoots.
- Clear, colorless baking pans make good heat traps when filled with water. They can be supported on two narrow boards over the light source.
- Place the shoot tips against the side of the cylinder so that the half that is not removed will remain in water.
- Shoot growth toward light is controlled by auxin transported from the apical meristem (Laferriere, 1993). With *Coleus blumei*, the effects of gravitropic responses are usually stronger than phototropic responses.
- Tomato seedlings also respond very quickly to gravity and can be easily handled in small pots.
- The results reported here were obtained with shoots 25 cm above a 55-watt incandescent bulb with 3 cm of water in the water trap. If a light meter is available, you may want to quantify the light and test different intensities or qualities of light.

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TEACHER'S NOTES

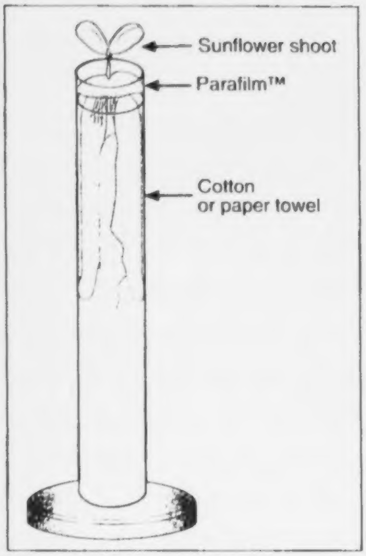

Sunflower shoot
Parafilm™
Cotton
or paper towel

Figure 10. Experimental setup.

4. Set up the control.
   a. In a dark cabinet, lay the control cylinders perpendicular to their original position.
   b. Tape the cylinder to the lab table. See Figure 11.

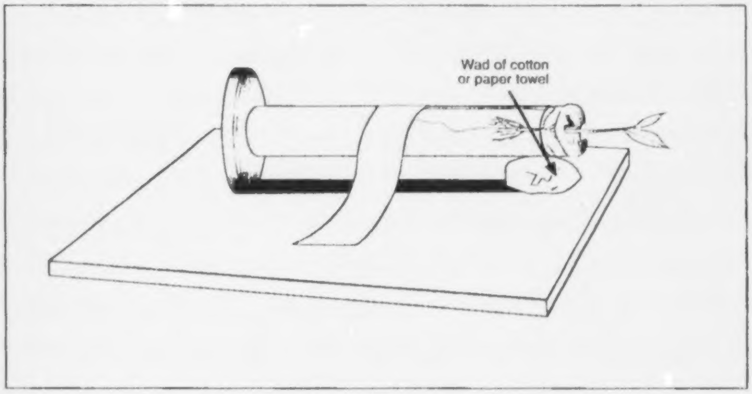

Wad of cotton
or paper towel

Figure 11. Graduated cylinder taped to the lab table.

5. Set up the treatment.
   a. Lay the treatment cylinder on the lab table in another dark cabinet.
   b. Immediately below the shoots, place a heat trap.
   c. Immediately below the heat trap, place the light facing upward.
   d. Turn on the light.
6. After 24 hours, measure the shoot angle from the horizontal. See Figures 12, a & b.

DATA ANALYSIS AND INTERPRETATION

Sample Data

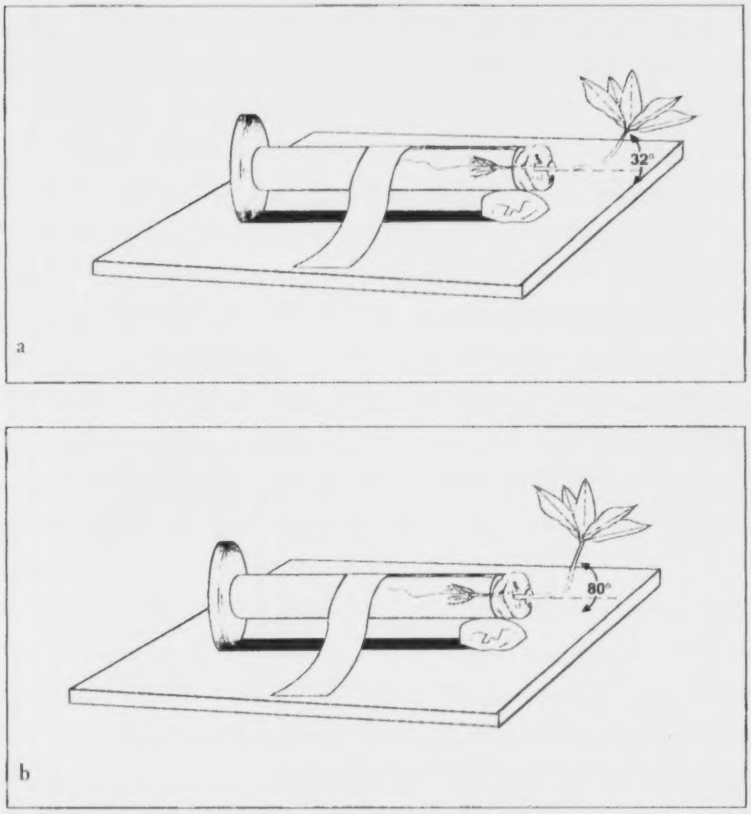


Figure 12 a & b. Stem position after 24 hours in the light (a) and in the dark (b).

Table 11. Sunflower stem angles after 24 hours exposed to gravity in the dark or lighted from below.

Replicate	Stem angle (°)	
	Light	**Dark**
1	32	80
2	30	84
3	30	84
4	34	86
5	30	80
6	32	80

An appropriate analysis for this data would be the Wilcoxon Two-Sample Test. Here, however, these data do not overlap and no statistical analysis is necessary. Draw diagrams of curved stems representing the average angle of curvature or chart the distribution of curvature angles.

TEACHER'S NOTES

Interpretation

The gravitropic response is stronger than the phototropic response because the angle of curvature is less for shoots lighted from below than for those only exposed to gravity.

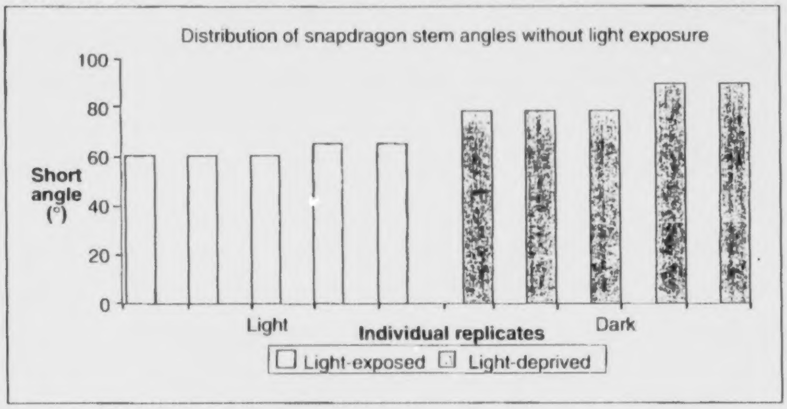
Answer to Test Question

These stems had rather uniform responses to the treatments. The direction of these responses is the same as those observed; the gravitational influence is stronger than the light response. The snapdragon stems were less responsive to light than were our sunflower stems because they curved less when exposed to the light.

VARIATION 8

TEST QUESTION

Compare the student data graphed below with the results of your experiment.



Graph F. Influence of light on the gravitropic response of snapdragon flowering stems.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have the student who is visually impaired note the setups used by sighted students, or team them with a sighted person if braille sketches of the positions of the cylinders and seedlings are not available.

VARIATION 8

The Effect of Removing the Shoot Tip on the Gravitropic Response of Sunflower Shoots

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will compare the gravitropic responses of sunflowers with and without the tip removed.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

☐ 1 single-edge razor blade or scalpel
☐ 10 straight sunflower stems with expanded leaves removed
☐ modeling clay
☐ 10 15-mL test tubes
☐ Parafilm™
☐ 1 damp paper towel

HYPOTHESIS GENERATION

Question

How will the gravitropic response of a sunflower be affected if the shoot tip is removed?

Sample Hypothesis

If the shoot tip of a sunflower stalk is removed, then the gravitropic response will be less.

Rationale

If the sensor for gravitropic response is in the roots, it is likely that the sensor for shoots will be in the tip.

Sample Experimental Procedure

1. Cut the stems of the 10 sunflower seedlings to the same length and insert them into test tubes filled with water.
2. Pack the opening of the test tube with damp paper towels or cotton to anchor the stem and seal the stem in with Parafilm™.
3. Divide the sunflower shoots into 2 matched groups of 5. One group will be the treatment group and the other, the control group.
4. Place the control plants on their sides with the shoot perpendicular to the floor. Use modeling clay or tape to wedge the test tube in a secure position.
5. Trim the shoot apex through the stem as close to the tip as possible.
6. Place treatment shoots on their sides with the shoot perpendicular to the floor. Use modeling clay or tape to wedge the pot in a secure position.
7. After 2 hours, measure and record the angle of curvature of each shoot. Figure 13 illustrates the angle to measure. Measure the angle formed by a line tangential to the curvature at the stem tip and a line parallel to the initial stem axis.

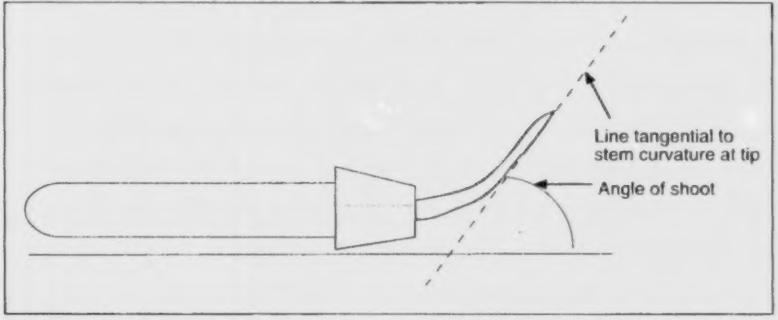


Figure 13. Diagram of technique for measuring the angle of shoot curvature.

DATA ANALYSIS AND INTERPRETATION

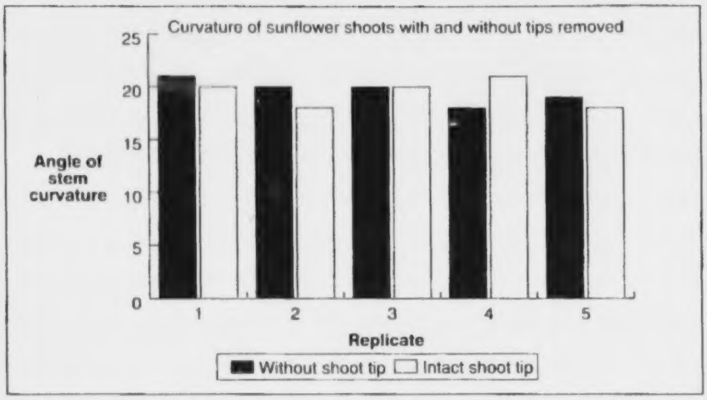
Sample Data

Table 12. Curvature of sunflower shoots with and without tips removed.

Replicate	Angle of stem curvature	
	Without tip	Intact shoot
1	21	20
2	20	18
3	20	20
4	18	21
5	19	18

TEACHING TIPS

- Shoots with intact leaves will produce the same responses. So much water is transpired by leafy shoots that they are likely to use all the available water and wilt.
- Sunflowers belong to the genus *Helianthus*. Small flowered kinds, like Jerusalem artichoke, *Helianthus tuberosus*, provide many useful shoots. You also can use seedlings started from sunflower seed intended for bird feed.
- Without leaves, the shoot will hold over for 24 hours, so the observations could be made the next day.
- The response will be stronger if the leaves are left on the stem, but the loss of water by transpiration will be great. If you want to do this with intact leaves, use only the short time period and replace the straw or narrow glass tubing with a 100-mL graduated cylinder or similarly large water reservoir.
- This response will be observed in a lighted classroom or in the dark. Students may want to perform the experiment in the dark to eliminate the possibility that they are observing a positive phototropism rather than a negative gravitropism.



Graph G. Effect of tip removal on the curvature.

Interpretation

Removing the shoot tip does not alter the gravitropic response of sunflower shoots. The mean control shoot angle was 19.6° and treatment angle height was 19.4°. These data for treatment and control almost completely overlap. The hypothesis is not supported.

TEST QUESTION

What is the adaptive advantage for a stem to exhibit a strong negative gravitropic response?

Answer to Test Question

The stem's main function is to display the leaves to the sun. It is adaptive for the leaf buds to be in a position for the expanding leaf to immediately receive sunlight rather than to have the leaf be the sensor for light or gravity. To orient the leaf buds correctly, the stem needs to grow toward the light before light is perceived. Even a shoot of a deeply buried seed should reach the light by negative gravitropism.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Use straws instead of test tubes with students who are visually impaired.

VARIATION 9

The Effect of Removing a Dandelion Flower on the Gravitropic Response of the Peduncle

***Note to Teachers:** In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.*

SYNOPSIS

Students will examine the effect of removing the flower from a dandelion on the gravitropic response of the peduncle.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- jumbo drinking straws or 6-mm diameter glass tubing
- modeling clay, cotton or paper towel
- 1 metric ruler
- 12 dandelion plants
- 12 15-mm test tubes
- 1 scalpel or razor blade

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Collect peduncles that are at least 15 cm long which are in the bud stage or stand erect and just beginning to disperse fruits. Keep them fully upright until they are to be used.
- Seal one end of the glass tubes with heat or seal one end of the straws with modeling clay.
- A syringe is useful for filling the glass tubing or straw with water.

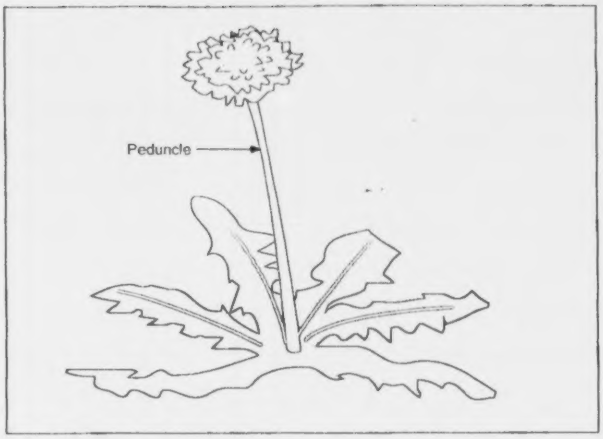


Figure 14. Dandelion plant.

HYPOTHESIS GENERATION

Question

Will the gravitropic response of the dandelion peduncle be affected if the bud of the flower is removed?

Sample Hypothesis

If the flower from a dandelion peduncle in bud stage is removed, the peduncle will not respond to gravity.

Rationale

The sensor for root gravitropic response is in the root tip so it seems likely that the sensor for the flower stalk will be at the tip.

Sample Experimental Procedure

1. Keep the peduncles upright while you work with them.
2. Fill 1 tube with water for each peduncle you have.
3. Place the peduncles into the tubes so that at least 10 cm extend above the tube. If the peduncles are dramatically different in length, cut them to about the same size by cutting off the lower portion. Retain the portion closest to the flower.
4. Gently anchor the peduncles so they cannot rotate in the tube by wedging in a little modeling clay, cotton, or tissue.
5. Divide your prepared peduncles into 2 groups. Leave the flower attached to the control peduncles. Cut the flower head from the treatment peduncles.
6. Lay the tubes on a counter so that the peduncles extend beyond the edge and anchor them in place with a small piece of modeling clay or tape.
7. After 3 hours, measure the perpendicular distance to the tip of the peduncle or the base of the flower from the plane of the counter on which its base is resting.
8. Analyze the growth of the peduncles.

TEACHING TIPS

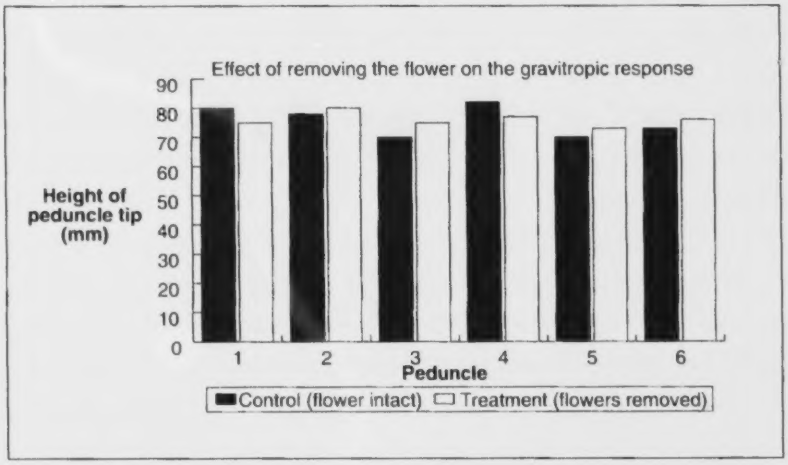
- A graduated cylinder makes a good carrying and storage container for dandelion flowering stalks (peduncles).
- The gravitropic response of dandelion peduncles varies with the stage of development (Clifford & Oxlade, 1991). Peduncles are strongly negatively gravitropic in bud, weakly gravitropic in flower, sometimes diagravitropic when the petals fall and the head closes during fruit maturation, and again strongly negatively gravitropic just before the fruits are dispersed.
- Expect a lag time of 20 to 30 minutes before the peduncle grows upward (Clifford & Oxlade, 1991).
- A 10-cm peduncle may become vertical in 2.5 hours (Clifford & Oxlade, 1991).
- The flower of a dandelion is a cluster of flowers in an inflorescence called a head. The scientific name for dandelion is *Taraxacum officinale*, Weber.
- Some flower stalks are strongly positively gravitropic or thig-motropic when their fruits develop. For example, peanuts (*Arachis*) are buried by the flower stalk, fruit axes of a relative of snapdragons (*Antirrhinum*) creep into fissures, and stems of a relative of rhubarb (*Polygonum*) drop seeds as they creep along the ground (Van der Pijl, 1972)
- Dandelion peduncles will continue to grow for several days if they are kept upright in water. See Figure 14.
- You can demonstrate that the bending of the dandelion peduncle is the result of differential growth by splitting the peduncle longitudinally, separating the top and bottom halves of a bent peduncle. You can measure a difference in the length of these sections. See Figure 14.
- A variation would be to study the height change over time by recording the height at half-hour intervals. There is usually a lag time in the appearance of the curvature.
- Weed seeds can be purchased from:
  Valley Seed Service
  PO Box 9335
  Fresno, CA 93791
  559.435.2163

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 13. Effect of removing the flower on the gravitropic response of dandelion peduncles after 3 hours.

Peduncle	**Height of peduncle tip (mm)**	
	Control (flowers intact)	**Treatment (flowers removed)**
1	80	75
2	78	80
3	70	75
4	82	77
5	70	73
6	73	76



Graph H. Flower removal effect on the gravitropic response.

Interpretation

Removing the flower does not alter the gravitropic response of the peduncle. The mean control peduncle height was 75.5 mm and treatment height was 76 mm. These data for treatment and control overlap almost completely. The hypothesis is not supported.

TEST QUESTION

Dandelion fruits are wind-dispersed. What is the adaptive advantage for having strong negatively gravitropic flower stalks in this species? Peanut fruits are buried by their flower stalks. Would you expect peanut flower stalks to be negatively gravitropic? Explain.

Answer to Test Question

For a plant with wind-dispersed fruits, erecting the flower stalk well into the air would be advantageous since it could elevate the fruits above surrounding vegetation and place them in a good launching location. Any plant, such as the peanut with its fruit or seed underground, could be expected to be positively gravitropic. A mechanism for orienting to the soil must be present.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Remind the student who is visually impaired that the peduncle is the stem. Drawings labeled in braille would be very useful.

Which Way to Grow

Directions for Students

INTRODUCTION

ROOTS CHASE THEMSELVES IN TIGHT CIRCLES! Could this be a news flash from the space station greenhouse? Could it be an everyday occurence there? Here we know this is not the common growth pattern. We might see it at the bottom of a potted plant that has outgrown its container, but the unrestrained growth of roots is normally different. Since the function of stems is to display leaves and the function of roots is to anchor and absorb water and nutrients, the upward orientation of stems and the downward orientation of roots is adaptive. Will plants orient correctly in space? What do you know about how plants perceive their surroundings? Which components of their surroundings do they respond to? Once they perceive the situation, how do you suppose they respond? Do all plants respond the same way? What does adaptive mean? Why are concerns about plant growth different in space than they are on Earth?

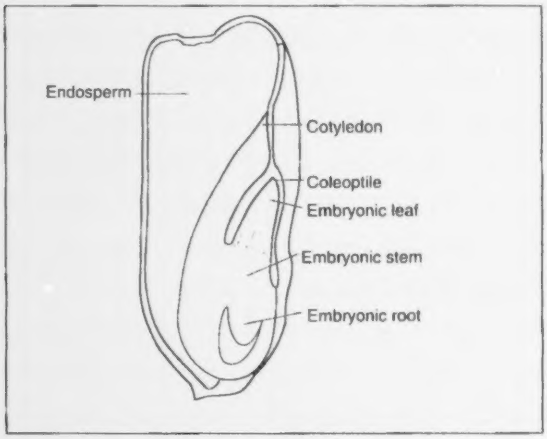


Figure 1. The structure of a corn seed.

OBJECTIVES

At the end of this lab, you should be able to:

- Explain how gravity is thought to cause the geotropic response in a corn plant.
- Examine the differences in gravitropic responses of corn variety or other plant species.
- Explain the influence of gravity, light, or touch on other plant organs.
- Describe the interaction of two environmental stimuli on plant growth.

SAFETY NOTE

Handle fungicide-treated seeds only with forceps or gloved hands.

MATERIALS NEEDED

For each team of two students, you will need the following:

- 1 package of mounting putty or 12 straight pins
- 12 presoaked corn seeds
- 4 pieces of 10-cm filter paper or 10 x 10-cm chromatography paper
- 1 plastic food storage box with lid
- 1 protractor
- 1 pencil

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STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of gravitropism and other tropic responses of plants. Summarize your findings and cite your references. The Internet is a rich resource for this topic because much of the research is government funded in connection with the space program. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. What makes this question an interesting or important topic for investigation?
3. What is the variable in your experiment?
4. What other variables will you need to control?
5. Why is controlling variables important?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. Combine your results with those of all your classmates. Construct a graph showing the relationship between the orientation of the embryo and the displacement angle of its root. Describe the relationship.
2. Do these data support your hypothesis? Discuss the evidence you used to come to this conclusion.
3. How could you create a variety of corn with the greatest gravitropic response you found among your tested kernels?
4. What other stimuli might help orient a root?

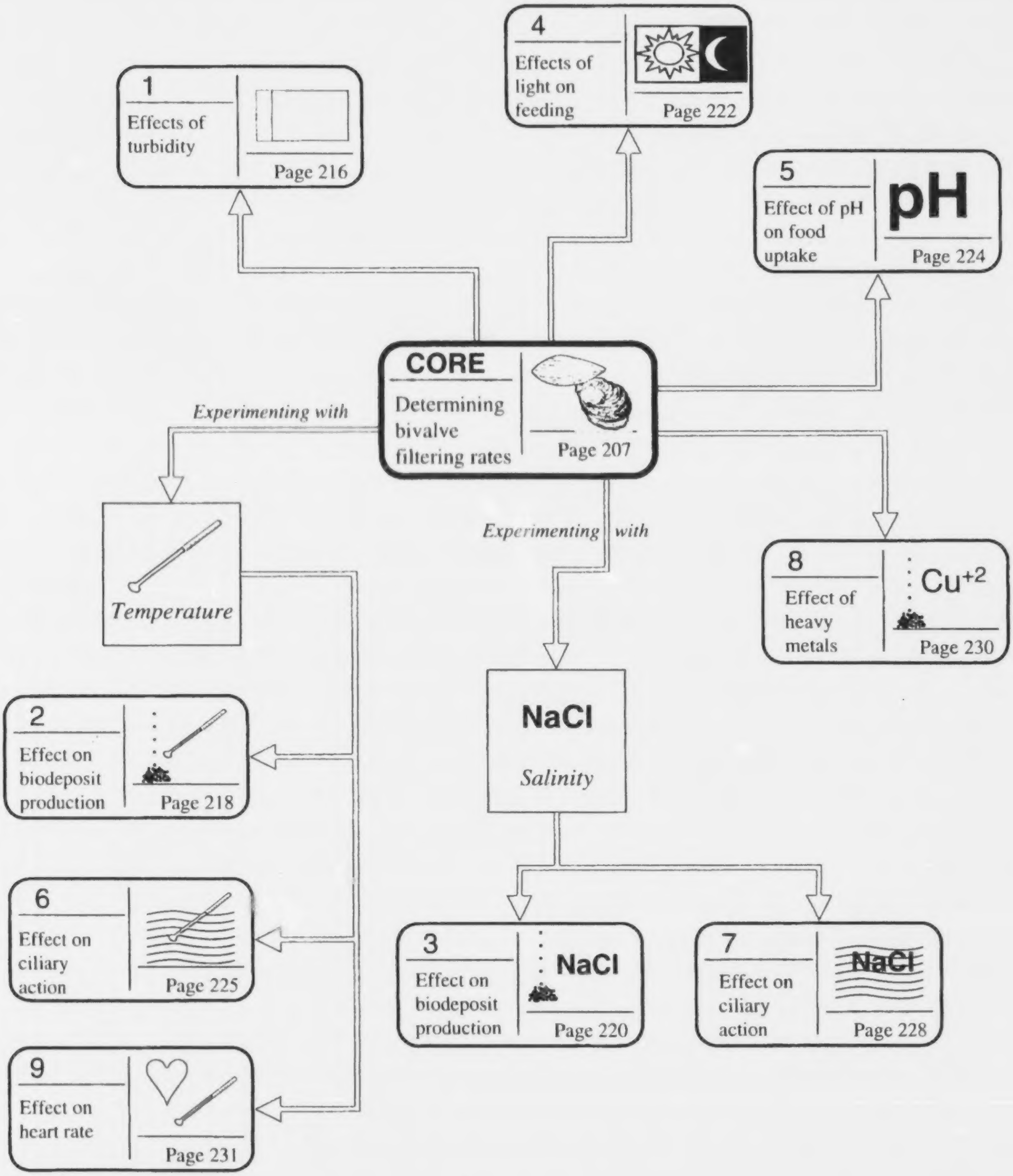
DESIGN OF VARIATIONS OF CORE EXPERIMENT

- What is the effect of increased temperature on the root growth angle of corn seedlings?
- What effect will light from below have on the gravitropic response of corn?
- How will corn roots respond to gravity if the water source is not below the roots?
- How is the gravitropic response of corn affected by the removal of its tip?
- Would failure to detect a difference in gravitropic response between different corn varieties have provided conclusive evidence that the response is not under genetic control?
- How will the addition of EDTA solution affect the corn root's response to gravity?
- Is the gravitropic response of sunflowers stronger than its phototropic response?
- How will the gravitropic response of a sunflower be affected if the shoot tip is removed?
- Will the gravitropic response of the dandelion peduncle be affected if the bud of the flower is removed?

202

The Impact of Mollusks on Water Quality

At-A-Glance Map of the Core Experiment and Its Variations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NOTES

The Impact of Mollusks on Water Quality

SYNOPSIS

Students will determine how a filter-feeding mollusk, such as an oyster, clam, or mussel, responds to changes in its immediate environment. Students will design experiments to test hypotheses about the effects of environmental variables on filter feeding and identify anatomical structures of a mollusk.

APPROPRIATE BIOLOGY LEVEL

Introductory, advanced, ecology

VARIATIONS 1 TO 9
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Directions for Teachers

Note to Teachers: Information is given for the Core Experiment below. Additional information needed for each variation of the Core Experiment may be found beginning on page 215. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Describe the ecological role of filter-feeding mollusks in fresh and/or saltwater ecosystems.
- Identify the major anatomical structures and functions of a filter-feeding mollusk.
- Describe how turbidity and salinity affect the behavior of a filter-feeding mollusk.
- Explain how a population of oysters, clams, or mussels impacts water quality.

MATERIALS NEEDED

For the teacher-led demonstration, you will need the following for a class of 24:

- 1 can of dead oysters or oysters in the shell from the fish market or store
- 1 live bivalve
- 1 algal culture
- carmine powder
- 1 eyedropper
- 1 handheld magnifying glass
- 1 light
- 1 video microscope (*optional*)
- 1 dissecting microscope (*optional*)

You will need the following for each group of four students in a class of 24:

- 5 live freshwater clams, such as the asiatic clam *Corbicula fluminea* or saltwater hardshell clams *Mercenaria mercenaria*, marine blue mussels *Mytilus edulis* or oysters, such as *Crassostrea virginica*

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LENGTH OF LAB

A suggested time allotment is as follows:

Day 1 (45 minutes)

- Demonstrate filtration and design experiment.

Day 2 (45 minutes)

- Set up experiment. Place animals into treatment aquaria.

Day 3 (20 minutes)

- Move animals from treatment tanks to collection containers.

Day 5 (45 minutes)

- Collect and discuss data.

PREPARATION TIME REQUIRED

Two weeks before lab:

60 minutes:

- Set up algal cultures and maintenance aquaria.

15 minutes:

- Set up the video camera (optional) and a shallow tray of water for the introductory demonstration.

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TEACHER'S NOTES

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- 0.5g (3t) kaolin powder
- 3 3.8-L (1-gallon) aquaria or wide-mouthed glass or plastic jars
- 12 L artificial seawater if using marine bivalves or 12 L nonchlorinated water, such as spring water if using freshwater bivalves
- 1 aerator
- 1 thermometer (°C)
- 15 petri dish halves
- 1 Pasteur pipette
- 10 10-mL graduated cylinders

SAFETY PROCEDURES

- Do not collect some species of freshwater mollusks. Some freshwater mussel species are endangered and many are restricted to single drainage systems.
- Have students wash their hands before and after the lab.
- Caution students not to pick up an aquarium containing water.
- Have students carry expensive glass instruments such as hemocytometers in unbreakable containers.
- Do not discard mollusks or wash water into local waterways, particularly if the mollusk used is not from the local area.
- When adding acid, remember to **add acid into water.**
- Wear safety goggles.
- Do not eat any of these organisms.

DIRECTIONS FOR SETTING UP THE LAB

- Expand the algal cultures by transferring 10 to 20 mL of culture to 1 liter of the appropriate culture medium. For freshwater bivalves, use selected algae of the Division Chlorophyta. *Ankistrodesmus* sp. or mixtures of *Ankistrodesmus* sp., *Chlamydomonas* sp., *Chlorella* sp., and *Scenedesmus* sp. are good food sources. The Bigelow Laboratory for Ocean Sciences does sell starter algal cultures. Contact information is as follows: Bigelow Laboratory for Ocean Sciences, McKown Point, PO Box 475, West Boothbay Harbor, ME 04575-0475; phone: 207.633.9600; fax: 207.633.9641. If you use the bivalve *Corbicula fluminea*, do not use *Selenastrum* sp. They are toxic to the asiatic clam (McMahon, 1991). For marine bivalves, use *Isochrysis* sp., a member of the Division Chrysophyta. Suitable concentrated culture medium for the freshwater species and culture medium for the marine species is available from Aquatic Research Organisms, Inc., PO Box 1271, Hampton, NH 03843-1271; 800.927.1650. The algae grow well at room temperature (22°C) and high light intensity for 12 to 16 hours/day. Use 2 x 4-foot fluorescent light fixtures or a similar setup.
- Set up a maintenance aquarium with aeration, filtration, and the temperature regulated at 18 to 25° C. Aquarium heaters can be used to increase the temperature above ambient. Provide a varied substrate with coarse sand or fine gravel and a few larger rocks. You may find it convenient to set up a smaller aquarium as a feeding station during experimentation. If you will be maintaining the bivalves, you will need to provide algae daily. Add sufficient algae to make the water turn just a very light green/brown. Use care not to overfeed.
- On the first day of this 3-day experiment, set up 3 small aquaria at room temperature with seawater, artificial seawater, or freshwater as appropriate.
- If fresh seawater is unavailable, use commercial instant seawater. Mix with rain, pond or river water, or with dechlorinated tap water to attain a salinity of about 35 parts per thousand (ppt).

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- Aerate the water in the aquaria with a small air pump without a filter, or manually aerate the water three or more times a day by dishing out and pouring back water to create bubbles.

TEACHER BACKGROUND

Content Information

Clams, mussels, and oysters are essential to the ecology as well as to the economic potential of the waters where they are found. Their population levels and health may indicate the condition of the water and the organisms found in it. They filter phytoplankton and other particulate matter from the water column and transfer it as biodeposits — feces and pseudofeces — to the sediment surface where it is a food resource for other aquatic animals. The removal of this matter allows for better light penetration in the water for benthic plants that are light-dependent.

These mollusks all filter water for feeding and respiration. Otherwise, their life habits are very different. Clams partially bury themselves in sand and clay, living as infaunal burrowers in relatively quiet water. Marine mussels attach to hard objects with byssus threads, living epifaunally in relatively agitated water. Oysters glue their left shell valve to rocks and other shells, living epifaunally cemented in relatively quiet water.

Filter-feeding mollusks are some of the first order consumers that feed on algae. They remove large amounts of these planktonic organisms from the water and thus participate in a natural cleansing process. Normally, they are able to assist in reducing the intensity of algal blooms. It has been estimated that the population of oysters living in the Chesapeake Bay was once so great that they could filter the entire volume of water in a few days, but they have declined so much that this process now takes over a year (Newell, 1988). In other areas, species of mollusks that have been introduced, like the asiatic clam, ***Corbicula***, and the zebra mussel, ***Dreissena polymorpha***, have proliferated to the point where special removal steps must be taken to prevent them from blocking the water intake pipes of water treatment plants and factories.

Chemical testing and biological assays reveal that human activities have influenced water quality of our rivers, lakes and estuaries significantly. One of the factors contributing to the deterioration of water quality is the disruption of the balance between producers and consumers. This disruption results, for example, in the algal blooms that occur during the summer in many waterways. The prolific growth of the algae is the result of large inputs of nitrates and phosphates. These nutrients come from agricultural runoff, sewage treatment outflows, or even acid rain.

A stable environment is necessary to the survival of all organisms and those that directly or indirectly depend upon them. Some species are unable to adapt even to slight changes in environmental factors, such as salinity, water temperature and movement, food source, oxygen levels. Other organisms are able to adapt and compensate within limits to changes in these factors. For example, organisms in an estuarine environment must be highly tolerant of fluctuating salinity levels.

In 1972, the impact of tropical storm Agnes' continued rainfall was observed not only in the Susquehanna watershed, but many kilometers away throughout the Chesapeake Bay. There were high mortality rates of organisms even in estuaries due to freshwater extending far into the estuaries. In addition, the low density freshwater covering the surface of the estuary prevented the water column from mixing to reoxygenate waters below. Without oxygen in the bottom waters, anaerobic bacteria replaced aerobic bacteria. Instead of oxygen, the anaerobic bacteria produced hydrogen sulfide gas that was toxic to other aerobic organisms. A change in one environmental factor had devastating effects for the entire ecosystem.

TEACHER'S NOTES

In addition to creating nutrient fluxes, rain and irrigation produce periodic changes in turbidity as the runoff carries a heavy sediment load. Where fires rage because smaller natural fires have been reduced or where lands have been cleared for development, the sediment load can be extremely high. Filter-feeding mollusks have a mechanism to clear some of the excess particulates, but when the concentration of suspended particles becomes high, the filtration rate slows and some species starve to death or suffocate.

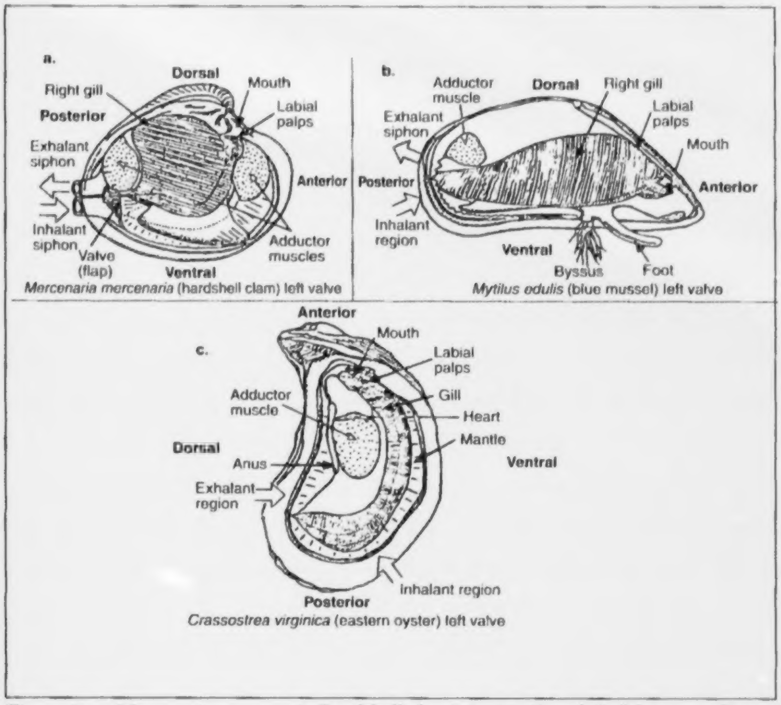
a.
Dorsal
Right gill
Mouth
Labial palps
Posterior
Exhalant siphon
Anterior
Inhalant siphon
Valve (flap)
Adductor muscles
Ventral
Mercenaria mercenaria (hardshell clam) left valve
b.
Adductor muscle
Dorsal
Right gill
Labial palps
Exhalant siphon
Mouth
Posterior
Anterior
Inhalant region
Ventral
Byssus
Foot
Mytilus edulis (blue mussel) left valve
Anterior
c.
Mouth
Labial palps
Adductor muscle
Gill
Heart
Mantle
Dorsal
Anus
Ventral
Exhalant region
Inhalant region
Posterior
Crassostrea virginica (eastern oyster) left valve

Figure 1. a. *Mercenaria mercenaria* (hardshell clam). b. *Mytilus edulis* (blue mussel). c. *Crassostrea virginica* (eastern oyster).

Bivalves create a current of water through their bodies by the beating of microscopic cilia on the surface of their gills and labial palps. See Figure 1. As water moves over the gills, inorganic and organic particles, which are their source of food, are caught by the gill cilia. They also may filter out the organisms responsible for paralytic shellfish poisoning. Particles larger than 2.0 to 3.0 µm are removed, but zebra mussels and asiatic clams are able to filter even smaller particles (1.0 µm). Non-nutritious particles are discarded as pseudofeces, while food particles are taken into the digestive tract. Pseudofeces are discarded by valve clapping. Indigestible fecal matter is bound together by mucus, released into a cavity near the exhalant siphon, and expelled on exhalant currents.

Many forms of pollutants are detrimental to the health of bivalves. These include organic sewage effluents, chlorine, and industrial effluents. Many times the health of the adult animal is not affected directly but their reproductive capacity is, and an unhealthy population results. Heavy metals may accumulate in their flesh. When the flesh is eaten by a predator such as a bird, a starfish, or even a human, the toxins are transferred to the predator. Many oyster beds have been closed to commercial harvesting due to potential fecal contamination. This has no adverse effects on shellfish, but may be a human health risk.

Phytoplankton and bacteria are major sources of food for oysters. One particular species of algae, *Isochrysis galbana*, recommended here as a lab food source, is a member of the Chrysophyta phylum. This species has two flagella at its anterior end and is a unicellular, motile sphere approximately 5 µm in diameter. The normal concentration of algae in natural waters may be in the range of several thousand per milliliter. In this lab, concentrations may reach 10^7 cells per milliliter.

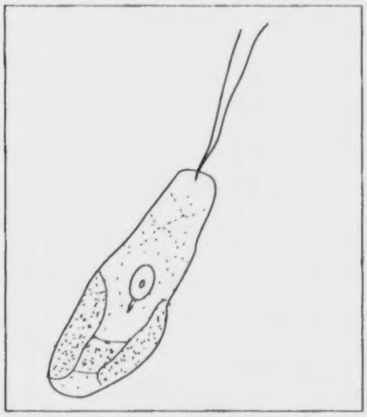

Figure 2. *Isochrysis* sp. (Newell & Newell, 1967).

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts:

Correct Concept	Misconception
Clear water is not a sufficient indicator of water quality. Other indicators such as chemical, bacterial, and pollutant counts provide much more useful information regarding the safety of water.	Good water quality means that the water is clear without concern for other parameters.
Filter-feeding mollusks play a major role in reducing turbidity in aquatic ecosystems.	Turbidity in aquatic ecosystems is reduced only by settling of particulate matter.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Obtain several live bivalves and demonstrate with carmine powder how water flows through the organisms. This can be done by using an eyedropper to transfer a drop of carmine powder mixed in a few milliliters of water to the water around the mollusk. Ask students what the organisms may add or remove from the water. Refer to Figure 1 to orient students to ventral and dorsal aspects. Bivalves produce two types of biodeposits — feces and pseudofeces — that in oysters are expelled on opposite sides of the organism. In bivalves with siphons, i.e. all clams and mussels, pseudofeces are ejected through the inhalant siphon and feces are expelled through the exhalant siphon. Identify the posterior and anterior ends of the specimen. Siphons or inhalant apertures are at the posterior end and labial palps are at the anterior end.

TEACHING TIPS

- Keep the list of hypotheses your students generate in class discussion. They may be helpful in the student design phase if a group is having difficulty.
- These data for this variation are presented as a scatter graph. They also could be presented as a bar graph of total deposits.
- A cross-curriculum project could be developed with the home economics or cooking courses.
- Living oysters and mussels are available from biological supply houses or seafood markets.
- If oysters are unavailable, hardshell clams or blue mussels may be substituted. If freshwater mollusks are used, set up freshwater instead of saltwater aquaria.
- If it is not convenient to have both clams and oysters, use one bivalve and study the filtering effect of increased numbers of bivalves. For example, place 3 bivalves in Aquarium 1 and 6 bivalves in Aquarium 2. Sample Data would be as follows:

Condition	Evidence of filtering	Biodeposit volume (mL)	Biodeposits per bivalve
3 Bivalves	A little	0 to 1	0.033
6 Bivalves	A lot	0 to 2	0.033
Unstocked	No	0 to 0	0.0

Interpretation

The greater the number of bivalves, the more material filtered from the water.

- Try to use different individual organisms for each variation.
- Feeding varies by body size. Try to reduce variation between treatments by matching animal sizes in treatment aquaria.
- Remove dead animals promptly because as they decay they foul the water quickly. If an animal's shell is more than 1 mm open, touch the animal lightly. If it does not respond by closing, attempt to pull open the valves. If they open easily, the animal is dead.
- Freshly collected animals can survive for weeks without eating. It is preferable to feed them before you begin the experiments. The best diet is mixed algae.

(continued on p. 208)

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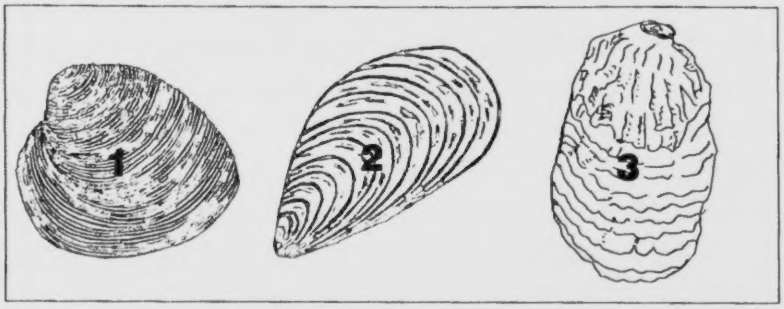

Figure 3. Shells of various bivalves should be labeled so that students can identify their specimens. Shell "1" is *Mercenaria mercenaria*, shell "2" is *Mytilus edulis*, and shell "3" is *Crassostrea virginica*.

Display a can of store-bought oysters to stimulate discussion of their food value. About 90% of food ingested by each successive stage in a food chain is respired, i.e., only 10% is available to be transferred to the next higher level in the food web. Marine producers, at least the phytoplankton, are difficult to harvest and process because of their small size and poor digestibility. As the second link in the food chain, marine bivalves that efficiently harvest from algal primary production provide a palatable, nutrient-rich animal food. Why are oyster bars sometimes declared unsafe? The most common hazard is the accumulation of bacterial pathogens. A natural hazard is the accumulation of dinoflagellate toxins. Industrial pollutants, both organic and inorganic, can accumulate to toxic levels (Boyle, 1981). Move to a discussion of the ecological role of shellfish as well as their economic role.

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses. Allow the students to reflect on the demonstration, then ask the following questions:

- What do oysters do that can be measured?
- What variables might affect these behaviors?

If necessary, you may suggest examples from the following list. Pair items from the first list with those from the second list and select possible hypotheses. Remind students of their knowledge of the bivalves so that their hypotheses have some rational biological basis.

Bivalve activities might include:	Variables might include:
Respire	Temperature
Eat	Light
Excrete	Salinity
Open and close shells	pH
Grow	Dissolved oxygen
	Toxic wastes
	Food type
	Food density
	Time of day
	Nature of substrate

TEACHING TIPS

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- Inexpensive aquaria may be constructed from plastic, 1-gallon milk containers or 3-L soda bottles by cutting off the tops. Ice cream, cottage cheese, or an assortment of other food storage containers also may make adequate treatment and collection containers. Closely monitor the temperature and water quality in these small containers as well as in the large ones.
- Wide-mouth, glass canning jars, 1-L beakers, or food storage containers make suitable "small collection containers."
- Substitute smaller aquaria for larger ones if your supply of seawater is limited.
- Kaolin is a form of clay used for ceramics and is available at pottery supply stores.
- Carborundum powder or a yeast suspension stained by heating with Congo Red may be substituted for carmine powder. All of these materials are likely to be rejected as pseudofeces after sorting, although some bivalves retain yeast. You also can use pureed frozen spinach with equal parts of water. If it clouds the water too much, rinse it first through a coffee filter, saving the particles on the filter. If you are using a saltwater bivalve, use saltwater to suspend your particles because freshwater floats on saltwater.
- Subtract the amount of material that settles in controls from experimental treatment amounts.
- If the combination of things you use does not show up well with the bright light and white background, try creating a dark field by putting a dark paper on the microscope stage, angling the light in from the side, and shielding the light from your eyes with a paper over the top.
- You do not actually measure filtration rate; you measure biodeposit production. In the oyster, *Crassostrea virginica*, no biodeposits form with 10 mg/L fuller's earth; at higher turbidity biodeposits form, but when turbidity is increased as high as 100 mg/L, water transport is depressed

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SAMPLE HYPOTHESES

- Oyster feeding rates will remain constant within a fixed range of salinities.
- Feeding rates will be greater in the dark than in the light.
- Heart rates will increase with a decrease in oxygen concentration.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 214.

Question

What role does filtering play in the life of a mollusk?

Hypothesis

Both oysters and clams actively remove algae from the water.

Rationale

Bivalves feed by filtering particulate matter from water. When exposed to increased turbidity, their filtering rate declines although the total amount of particles removed from the water will increase. However, when a mollusk takes in too much inedible particulate matter, it will impact negatively on the life functions of the mollusk. The result will depend on the relative weight and size of the oysters and clams.

Procedure

Day 1

1. Place 3 small marine aquaria with artificial seawater in a dark, quiet place.
2. Aerate the water in the aquaria manually by dishing out and pouring back water to create bubbles, 5 or more times daily. Include when you first arrive in the morning and before you leave school each day.

Day 2

1. Place 1 each of 5 small, healthy clams in one half of a petri dish. Place the petri dishes in 1 aquarium. Repeat this procedure with 5 small, healthy oysters in a second aquarium, and with 5 empty clam or oyster shells for the control in a third aquarium. See Figure 4.
2. Acclimate to room temperature for 1 to 2 weeks.

TEACHING TIPS

(continued from p. 208)

by 50%. Some oysters can tolerate up to 700 mg/L silt (Jørgensen, 1966).

- If only small amounts of biodeposits are produced, a measuring device may be made from a tuberculin (1-mL) syringe. Plug the attachment point for a needle with a piece of pipe cleaner or cotton. The syringe can be cleaned and reused. A serological pipette graduated to the tip also makes a good substitute for a small graduated cylinder.

TEACHER'S NOTES

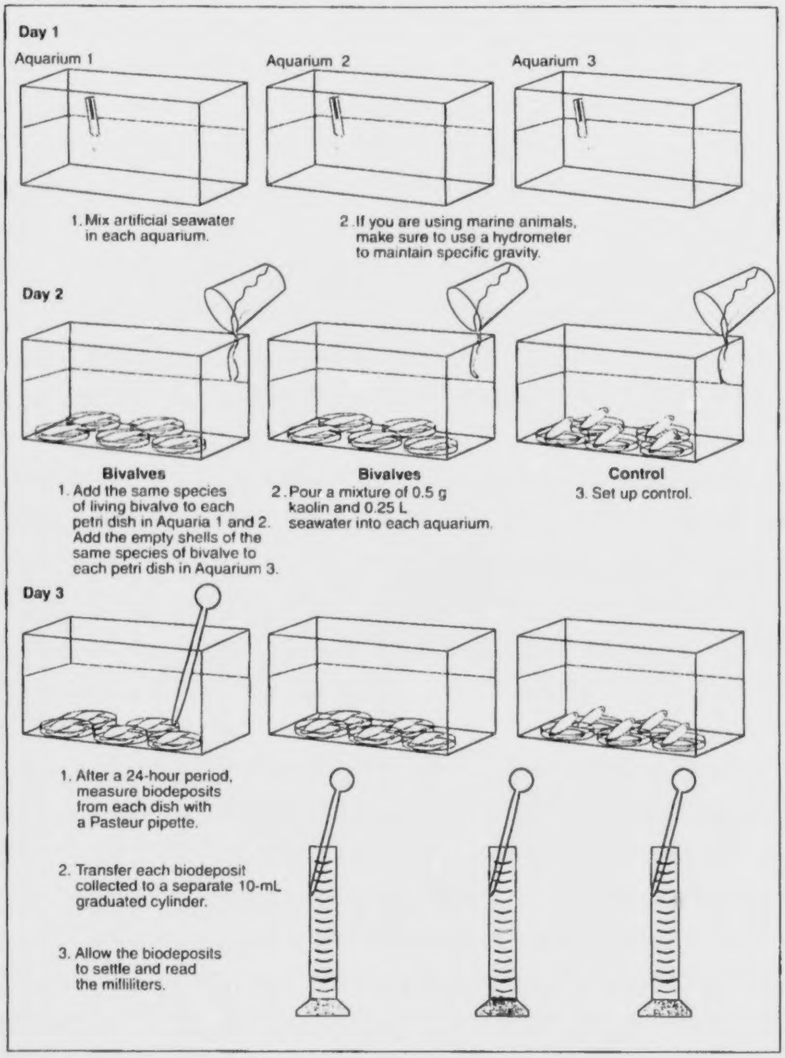

Day 1
Aquarium 1
Aquarium 2
Aquarium 3
1. Mix artificial seawater in each aquarium.
2. If you are using marine animals, make sure to use a hydrometer to maintain specific gravity.
Day 2
Bivalves
1. Add the same species of living bivalve to each petri dish in Aquaria 1 and 2. Add the empty shells of the same species of bivalve to each petri dish in Aquarium 3.
Bivalves
2. Pour a mixture of 0.5 g kaolin and 0.25 L seawater into each aquarium.
Control
3. Set up control.
Day 3
1. After a 24-hour period, measure biodeposits from each dish with a Pasteur pipette.
2. Transfer each biodeposit collected to a separate 10-mL graduated cylinder.
3. Allow the biodeposits to settle and read the milliliters.

Figure 4. Experimental design setup.

One week later

1. Add a mixture of 0.5 g of kaolin mixed in 0.25 L of seawater to each of the 3 aquaria. Stir the water gently to homogenize.
2. Keep all 3 aquaria in a dark, quiet place and allow the bivalves to filter the water, undisturbed, for 24 hours.

Next day

1. After the 24-hour period, examine the water in each of the petri dishes in the 3 aquaria for evidence of filtering to determine the presence of fecal and pseudofecal pellets.
2. Measure the biodeposits produced by each clam and oyster using the following procedure:
   a. With a Pasteur pipette, remove the biodeposits produced by each organism from the petri dish.
   b. Transfer the biodeposits produced to separate 10-mL graduated cylinders.
   c. Allow the biodeposits to settle and read the number of milliliters on the cylin-

cylinder. This number gives a crude measure of biodeposits produced that is an indirect measure of filtration and feeding rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Condition	Evidence of filtering	Biodeposit volume
Clams	Only a little	> 0.1-mL
Oysters	Yes	0.2-mL
Unstocked	No	0.0-mL

TEST QUESTION

Why would you expect extremely high concentrations of suspended particles to reduce filtration rates? Do your data support a reduction in rates at high concentrations?

STUDENT DESIGN OF THE NEXT EXPERIMENT

After students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for additional experiments. They should think of questions that occurred to them as they conducted their first experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- How will factors such as temperature, pH, or salinity affect biodeposition rates?
- Do the rates of biodeposit production differ among oysters, clams, and mussels?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

- Have the student who is visually impaired do library research on a scientist, such as Dr. Geerat Vermeij. Dr. Vermeij has been blind since the age of 3 and is well known for his ability to identify mollusks simply by the use of touch (Vermeij, 1996b). Sighted students may wish to identify different species of mollusks provided by the instructor by using just the sense of touch.
- Prepare raised-line drawings or braille paintings of the cross section of the mussel, clam, and oyster shown in Figure 1 of the Core Experiment. Enlarge each mollusk to the size of a sheet of braille paper. See Figure 5.

Interpretation

Oysters are more productive at filtering particulate matter from the water.

Answer to Test Question

Filter-feeding bivalves depend on ciliary filtering and sorting. If the filtering mechanism is overwhelmed by sediment, the organism will be unable to obtain adequate oxygen or adequate nutrients. Shutting down the filtration system while suspended particle density is high could be more efficient than continuing to clean debris from the gills. Many bivalves are unable to survive in waters with high silt. These data do not support the hypothesis that there is a reduction in filtration rate at high concentrations of suspended particles because only one particle concentration was tested. At least two concentrations are needed to test this hypothesis.

TEACHER'S NOTES

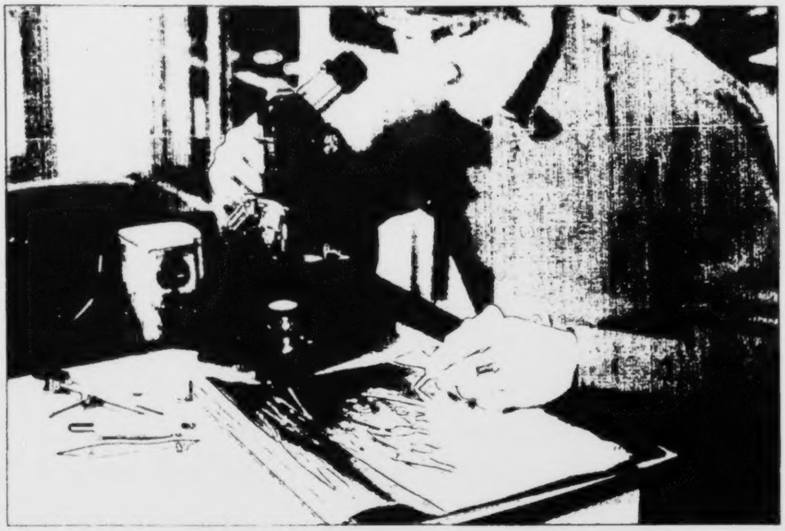


Figure 5. Making a raised-line drawing with a stylus on braille graph paper.

- Transfer a drop of carmine powder in solution to the water around the mollusk. Visually impaired students may be able to detect the path of the carmine in the gills with a light sensor.
- Provide students who are blind with a variety of mollusks to understand the differences in the animals used in this activity.
- Have a sighted lab partner remove the biodeposits produced by each mollusk with a pipette and place them in a 10-mL plastic graduated cylinder with raised lines for volume amounts. The student who is visually impaired can use a thumbnail or plastic card to determine the number of spaces indicating volume. With a light sensor, the student can determine the amount of pseudofeces produced by locating the line that separates the pseudofeces from the liquid. See Figure 6.

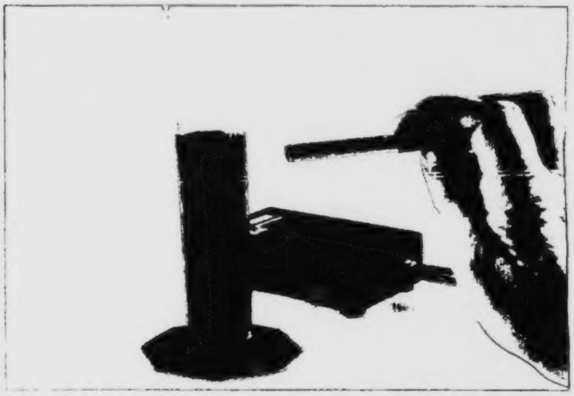


Figure 6. Using the light probe to determine liquid level in a plastic graduated cylinder with raised lines for volume amounts. Accuracy from ± 1 or 2 mL varies with the experience of the operator and the type of probe used.

Deaf or Hard-of-Hearing

There are no specific concerns related to this investigation for the hearing impaired. Evaluate the communication skills within the groups containing members who are deaf.

Gifted

Have the students who are gifted do independent research on the local mollusks, gathering information for a class report on the population, habitat characteristics, life cycle, and ecology of the organisms. They could contact mentors from local universities, museums, or shell-collecting clubs and work on ways to use computerized data collection methods both in the lab and in the field.

TEACHER'S NOTES

Mobility Impaired

- Provide a comfortable position for microscope work for students in wheelchairs. If counters in the laboratory are too high, consider transferring the student to a platform chair that is secured to a 20 x 24-inch wooden platform with sturdy caster wheels. See Figure 7.

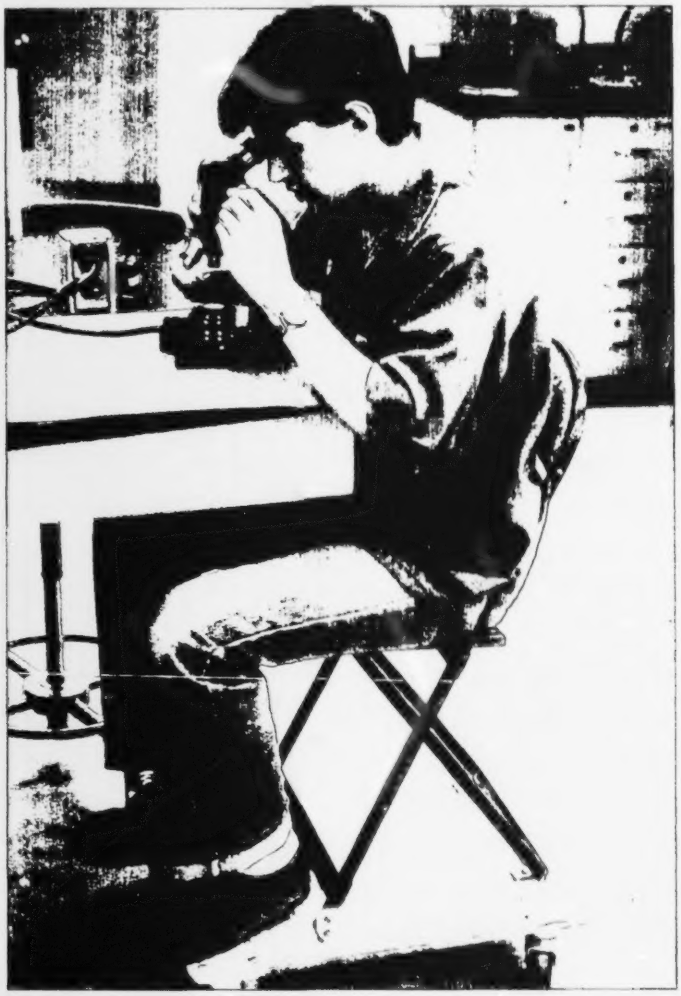


Figure 7. Wheelchair platform for microscope work.

- Offer students with only one arm access to clipboards or other available clamping devices that hold objects or writing materials.
- Offer students who are palsied access to a microscope that has been secured to the counter. A frame that accommodates a microscope can be fastened to the lab table.
- Provide students who are palsied with a guard on the oculars to steady their heads

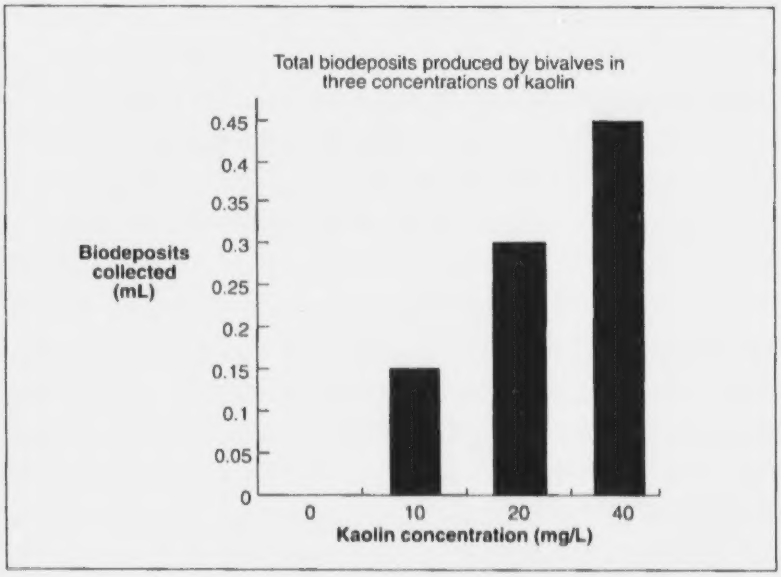
while using a microscope. The small, inexpensive rubber shields such as those used on cameras to keep out stray light work well.

- Provide a bar on the microscope's fine and course adjustments that will allow them to be turned easily.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment may include the following:

1. Identify the independent and dependent variables in this experiment.
2. Identify the limitations in this experiment in terms of number of trials and explain how the experiment could be improved.
3. Design an experiment to determine the effect of temperature on the ability of oysters to remove particles from the water.



Graph A. The production of biodeposits by bivalves exposed to different concentrations of kaolin.

Answers to Additional Test Questions

1. The dependent variable is total biodeposits; the independent variable is the number of bivalves. The number of bivalves is the independent variable because it can be controlled by the investigator, and the amount of biodeposits produced is dependent on the total number of animals used in each experiment.
2. All animals exhibit individual variation. In order to smooth out this variability, at least 3 aquaria per treatment should be used, i.e., increase replication.
3. Use one turbidity level, a moderate value, and expose 3 to 5 bivalves to varying temperatures from 5 to 30°C. Measure the change in feeding rate with each temperature.

Answers to Questions and Analysis on Student Page

1. The answers will vary depending on the students' data. The graph title and axes should be similar to those in Graph A.
2. These data support the hypothesis that more biodeposits will be produced when the water is turbid.
3. Doubling the amount of suspended material, kaolin, resulted in a doubling in the amount of biodeposits produced. Compare 10 and 20 mg/L.
4. Results will vary. Students may comment on small sample sizes, inadvertent temperature differences, or student techniques.
5. The amount of biodeposits produced under the identical conditions may vary depending on the physiology of the organisms.
6. The overall health of the animals may influence the rate of filtration, and consequently the amount of biodeposits produced.

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POSSIBLE SOURCES OF MENTORS

National Marine Fisheries Service, Milford, CT 06460

Access shellfish groups on the Internet and ask for a response from a nearby scientist.

VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

***Note to Teachers:** Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.*

TEACHER'S NOTES

TEACHER'S NOTES

VARIATION 1

The Effect of Turbidity on Bivalve Filtering Rates

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 1.

SYNOPSIS

Students will compare the effects of increased turbidity on bivalve filtering rates.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24:

- 12 live bivalves, such as eastern oysters (*Crassostrea virginica*), mussels (*Mytilus edulis*), or asiatic clams (*Corbicula fluminea)*
- 1 permanent marker
- 1 scrub brush
- 5 treatment aquaria
- 20 L natural or artificial seawater or 20 L freshwater
- 12 small collection containers
- 2 g kaolin
- 1 thermometer (°C)
- 5 air lines
- 5 air stones
- 1 aquarium pump
- 1 Pasteur pipette with bulb or transfer pipette
- 4 10-mL graduated cylinders

HYPOTHESIS GENERATION

Question

How does the influence of particle concentration on feeding rate differ between different species of bivalves?

Sample Hypothesis

Bivalve filtering rates will increase as the turbidity increases within a fixed range.

Rationale

There is an optimum filtering rate with increasing turbidity. At some level "x," the bivalve will not be able to filter at a capacity that will maintain a healthy state, and the organism may suffocate.

Sample Experimental Procedure

1. On the first day of the experiment, set up 4 treatment aquaria with water like that in the maintenance aquarium described in the Core Experiment.
2. Aerate and heat the water to the same temperature as the maintenance aquarium. Set the treatment aquaria in a place to maintain temperatures.
3. Add kaolin to the containers to produce the concentrations shown in Table 1. Make sure students know that kaolin is not a food source for the mollusks. Kaolin must be added to a few milliliters of tap water to produce a slurry before dissolving it in a larger volume of water. Remember to use dechlorinated water as the base for freshwater organisms and seawater as the base for marine organisms. If supplies of lab materials are limited, each group of students can set up the experiment with a different container. Class data may be pooled.

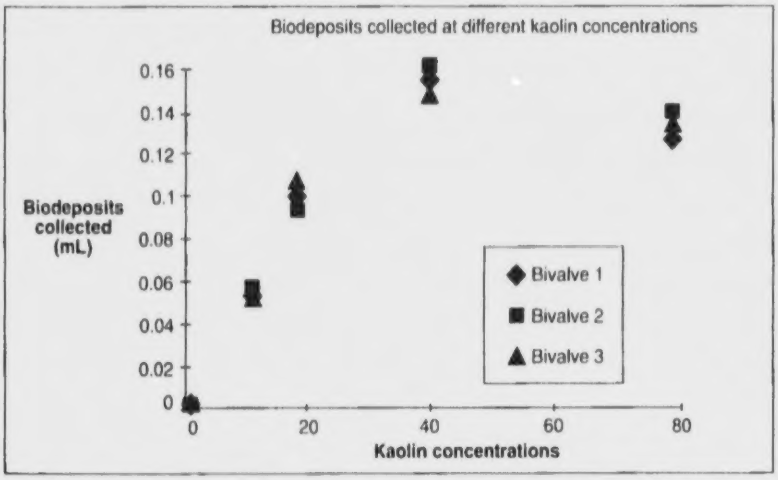
Table 1. Setup for Variation 1.

Treatment aquarium	Number of bivalves	Kaolin concentration (mg/L)
1 (Control)	3	0
2	3	10
3	3	20
4	3	40
5	3	80

4. Day 2, obtain 12 healthy bivalves. Wash each organism with a scrub brush under running tap water, pat dry, and number with a waterproof marker.
5. Place 3 bivalves in each treatment aquarium.
6. Check all organisms to make sure they are producing biodeposits. If some are not functioning, replace them with others.
7. Leave the organisms in the treatment aquaria for 24 hours.
8. After 24 hours, transfer the organisms to individual small collection containers with the same conditions as the maintenance aquarium. Keep records of which organisms were in each treatment aquarium.
9. After 48 hours in the small collection containers, measure the biodeposits produced by each organism.
    a. With a Pasteur pipette, remove the biodeposits produced by each organism from the small collection container.
    b. Transfer the biodeposits produced by each organism to separate 10-mL graduated cylinders or 15-mL graduated centrifuge tubes.
    c. Allow the biodeposits to settle and read the number of milliliters on the measuring container. This number gives a crude measure of biodeposits produced, which is an indirect measure of the filtration or feeding rate.
10. Return the organisms to the maintenance aquarium. Pool the class data.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph B. The volume of biodeposits produced in 48 hours by freshwater bivalves incubated for 24 hours in turbid water. These data are hypothetical, but fit the pattern expected with increasing turbidity and are of the magnitude reported in some student experiments.

TEACHER'S NOTES

Interpretation

More deposits were produced when the amount of suspended material was increased. The relationship was not linear. At the highest kaolin concentration less material was deposited than expected. The bivalves may reduce their filtration rate when the turbidity is very high.

TEST QUESTION

Why would you expect extremely high concentrations of suspended particles to reduce filtration rates? Do your data support a reduction in rates at high concentrations?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Use a Talking Thermometer to check the temperature of the water in each aquarium.
- Provide a balance with a taped midpoint for the student who is blind to locate the pointer as it comes to rest. Blind students can measure the amounts of kaolin. The weights on the beams slide into notches and are counted by touch to determine the value. For the balance slide with no grooves, the student's braille ruler can be calibrated with the scale.
- Have the student who is visually impaired prepare graphs of data on braille graph paper. The bars may be filled in with a crayon or tool that gives a raised line.

VARIATION 2

The Effect of Temperature on Bivalve Production of Biodeposits

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will measure and compare the production of biodeposits from bivalves held at different temperatures.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- 1 thermometer (°C)
- 2 aquarium heaters

HYPOTHESIS GENERATION

Question

How will temperature affect the production of biodeposits?

Sample Hypothesis

The production of biodeposits by bivalves will increase with increasing temperature.

Rationale

An increase in temperature typically results in an increase in metabolic rate. Increasing the metabolic rate should require additional food intake which could be accomplished by faster filtration rates. Faster filtration would result in the production of more biodeposits.

Sample Experimental Procedure

1. Use the kaolin concentration for which you observed the highest rate of biodeposit production in the linear part of the curve from the Variation 1 experiment. In the hypothetical data for the Variation 1, it would be 20 mg/L.

Answer to Test Question

Filter-feeding bivalves depend on ciliary filtering and sorting. If the filtering mechanism is overwhelmed by sediments, the organism would be unable to obtain adequate oxygen or adequate nutrients. Shutting down the filtration system while suspended particle density is high could be more efficient than continuing to clean debris from the gills. Many bivalves are unable to survive in waters with high silt. Our data support a reduction in filtration rate at high concentrations of suspended particles.

TEACHING TIPS

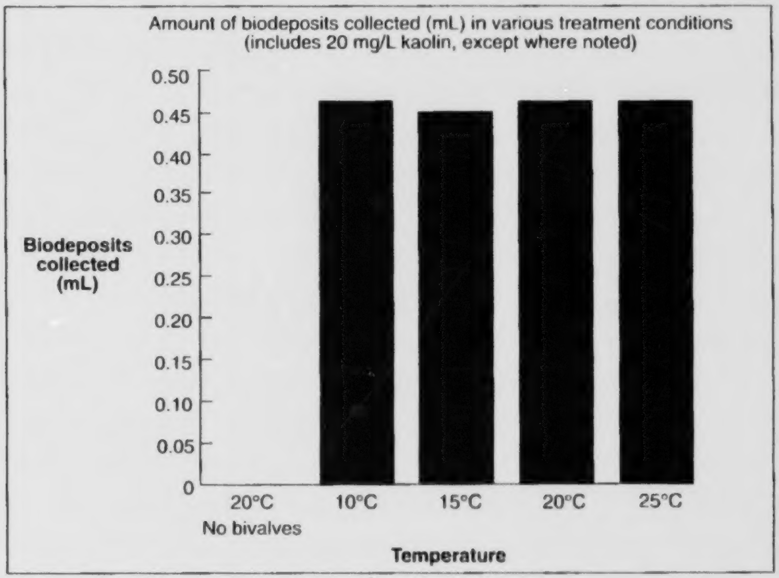
- Begin the experiment immediately after putting the organisms in the new temperature. An immediate temperature change is expected to cause an abrupt metabolic change in ectothermic animals. Bivalves show seasonal variation related to both temperature and reproductive cycles. If the organisms are allowed to acclimate to the new temperatures, they may show no change in metabolism, show the expected immediate change in metabolism, or show a reverse metabolic response (McMahon, 1991). Bivalves are very diverse in their temperature responses.
- Metabolic activity can be suppressed by decreased oxygen availability, so be certain to maintain aeration in all aquaria.
- Although different species have specific upper and lower temperature limits for survival and reproduction, temperatures of 2 to 25°C are probably safe for most bivalves.
- Sudden large temperature changes may be lethal. Ten degrees above and below the maintenance aquarium are likely to give good results. This may mean using fewer treatment aquaria than you used for the Core Experiment.

(continued on p. 219)

2. Maintain the temperature of 1 aquarium and the control aquarium, i.e., shells only to account for the passive settling of the kaolin, at the same temperature used in the maintenance aquarium.
3. Maintain constant temperatures 5 to 10°C different than the maintenance aquarium in each of the other 2 aquaria.
4. Repeat the incubation and data collection procedure of Variation 1.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph C. Relationship between biodeposit production and temperature. Note that at 20°C, there is no kaolin.

TEST QUESTION

A student hypothesizes that bivalve filtration rates will increase with increasing temperature. Do the following data support that hypothesis? What biological explanation might account for these data?

Table 2. Biodeposit production at two temperatures.

Replicate	Biodeposits (mL) collected at	
	10°C	20°C
1	1.5	2.2
2	1.7	2.0
3	1.4	1.9
Mean	1.53	2.03

TEACHING TIPS

(continued from p. 218)

- Some freshwater clams of the genera *Sphaerium*, *Musculium*, and *Corbicula* do not show significant correlation between filtering rate and temperature (McMahon, 1991).
- To maintain a temperature of 5°C, put the aquarium in a cooler on ice.
- In the oyster, *Crassostrea virginica*, optimum temperature for water transport is around 20°C.
- The control in this experiment should be dead bivalves, i.e., shells only, to account for the passive settling of the kaolin.

Interpretation

Accept the hypothesis if more biodeposits are collected at high temperatures than at low temperatures. This would suggest that metabolic activities associated with filtration are sensitive to environmental temperature as you would expect in a ectotherm. Reject the hypothesis if there are fewer or the same amount of biodeposits collected at higher temperatures than at lower temperatures. This would suggest that some other factor controls the rate of filtration.

Answer to Test Question

These data support the hypothesis that the filtration rate will be greater at a higher temperature because 0.5 mL more biodeposits were produced at the higher temperature. Metabolic rate usually increases with increasing temperature. The increase could be related partly to the increased rate of enzymatic functions at higher temperatures. This rate is less than an expected doubling, but still supports the hypothesis.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See the Core Experiment and Variation 1.

VARIATION 3

The Effect of Water Salinity on Oyster Biodeposit Production

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will measure and compare the biodeposit production of oysters or other marine mollusks held at different levels of water salinity.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- Instant Ocean™
- 1 balance
- 1 weigh boat
- 1 scoop
- 1 1-L graduated cylinder

HYPOTHESIS GENERATION

Question

Are oysters adaptable to a range of salinities?

Sample Hypothesis

Within a range of salinities, oyster feeding behavior will be constant.

Rationale

Living in an estuarine environment requires the ability to adapt to ever-changing conditions of salinity.

Sample Experimental Procedure

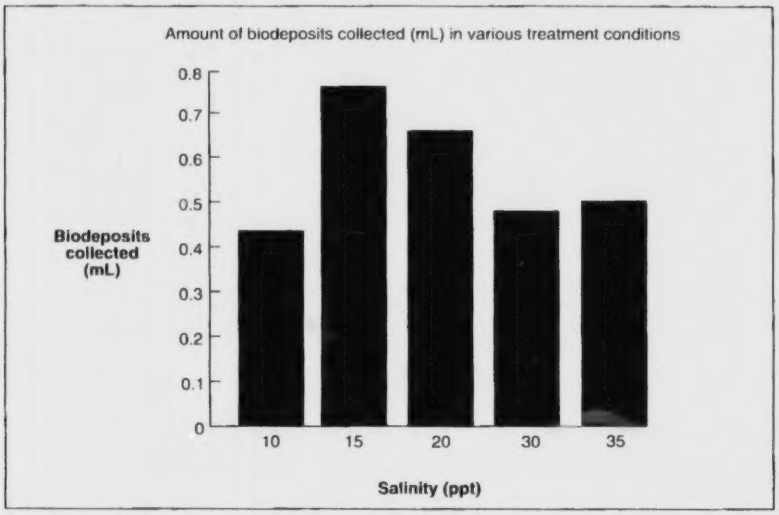
1. Use the kaolin concentration for which you observed the highest rate of biodeposit production in the linear part of the curve from the Variation 1 experiment. In the hypothetical data for Variation 1, this is 20 mg/L.
2. Maintain the salinity of 1 aquarium and the control aquarium with only bivalve shells at the same salinity used in the Core Experiment. In each of the other 3 aquaria, use 10 to 35 ppt.
3. Allow the bivalves to acclimate to their saline environments for 24 hours before adding the kaolin. Incubate and collect data as in Variation 1.

TEACHING TIPS

- For the oyster, *Crassostrea virginica*, salinities from 25 to 39 ppt resulted in normal pumping activity, 20 ppt in slower pumping, and below 13 ppt no pumping (Jørgensen, 1966).
- Use artificial seawater salts rather than sodium chloride, because ion imbalances with sodium chloride may result in the death of animals (Dietz et al., 1996).
- Provide background on estuarine communities if students have not observed this community previously.
- Salts to make seawater of different concentrations, such as Instant Ocean™, are available from most pet stores.
- Note that only marine bivalves can be used in this variation.
- The control in this experiment should be just shells to account for the passive settling of the kaolin.

DATA ANALYSIS AND INTERPRETATION

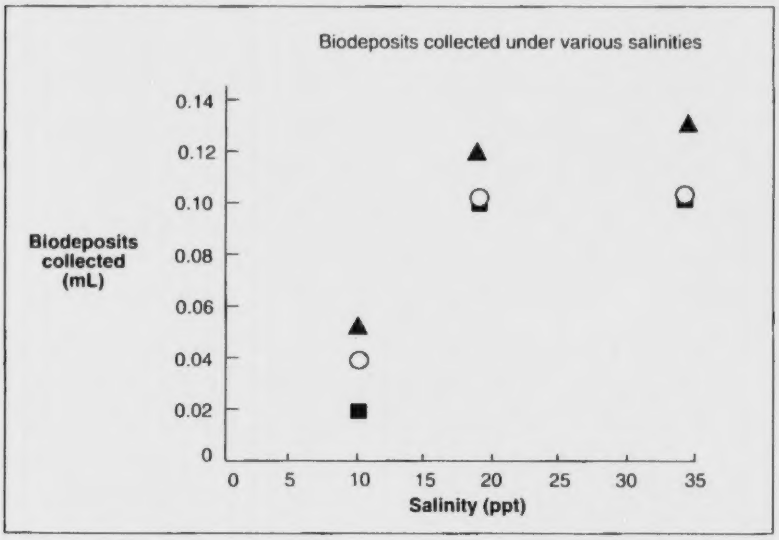
Sample Data



Graph D. Relationship between salinity and biodeposit production. Note that at 30 ppt, there are no bivalves and no kaolin. The control in this experiment is just shells to account for the passive settling of the kaolin.

TEST QUESTION

These data in Graph E represent the results of a student experiment with oysters in 3 aquaria of different salinities with a constant amount of kaolin. The student had hypothesized that there would be no difference in the production of biodeposits with different salinities. Do these data support the hypothesis? How would you answer this question if the student had only collected data at 25 and 35 ppt?



Graph E. Biodeposit production at different salt concentrations.

Interpretation

The hypothesis should be accepted if the amount of biodeposits is constant over the range of tested salt concentrations. This would mean that the bivalves filter equally under all tested salinities. The hypothesis should be rejected if the amount of biodeposits varies with salinity.

Answer to Test Question

These data show increased production of biodeposits at the two highest salinities. Reject the hypothesis because it predicts equal amounts of biodeposits at all salinities. If this student had only measured biodeposits at the two highest salinities, one would have accepted the hypothesis for that range of salinities because the amounts collected were the same.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired
- See Variation 1.

VARIATION 4

The Effects of Light on Feeding Activity

Note to Teachers: In addition to the information found in Variation 1, the following material has been provided for Variation 4.

SYNOPSIS

Students will compare the disappearance of algae in the aquarium water in the light and the dark.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- 1600 mL culture containing a thriving algal culture (*Isochrysis*, sp. for marine mollusks, *Ankistrodesmus* or mixed culture for freshwater mollusks)
- 8 aquaria
- 1 hemocytometer slide (see Figure 8) and cover slip
- 1 Pasteur pipette with bulb
- 1 compound microscope

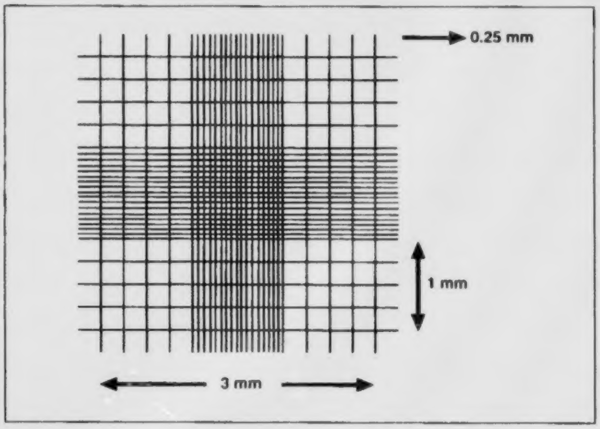


Figure 8. Hemocytometer.

SAFETY PROCEDURE

Carry expensive, breakable instruments like hemocytometers in unbreakable containers.

HYPOTHESIS GENERATION

Question
What effect does light have on the filtering rates of bivalves?

Sample Hypothesis
The rate of clearing of algae from the water by bivalves will be greater in the dark than in the light.

TEACHING TIPS

- Use a video microscope to introduce the Neubauer hemocytometer shown in Figure 8. Explain the method of counting the 4 corner 1-mm squares and the center 1-mm square, dividing that number by 5 to estimate the number (n) of cells per 1-mm square. Because the depth of the counting chamber is 0.1 mm, the number of cells per 1 mm^3 equals n x 10. In order to express this as cells per mL, multiply by 1000. Put the algal culture on the slide and have students determine the density of the culture.
- Some observations of *Crassostrea* show very little retention of low concentrations of algal cells unless some other particulate matter is present in the water, such as kaolin to increase particle concentration (Jørgensen, 1966).
- You should be able to detect differences in algal concentration at levels too low to show pseudo-feces formation.
- Dark can be created by covering the aquaria or putting them in a cabinet. Be certain, however, to keep all water aerated.
- If you do not have a hemocytometer, you can count cells per microscope field to obtain comparative data. Be certain everyone uses the same magnification.

Rationale

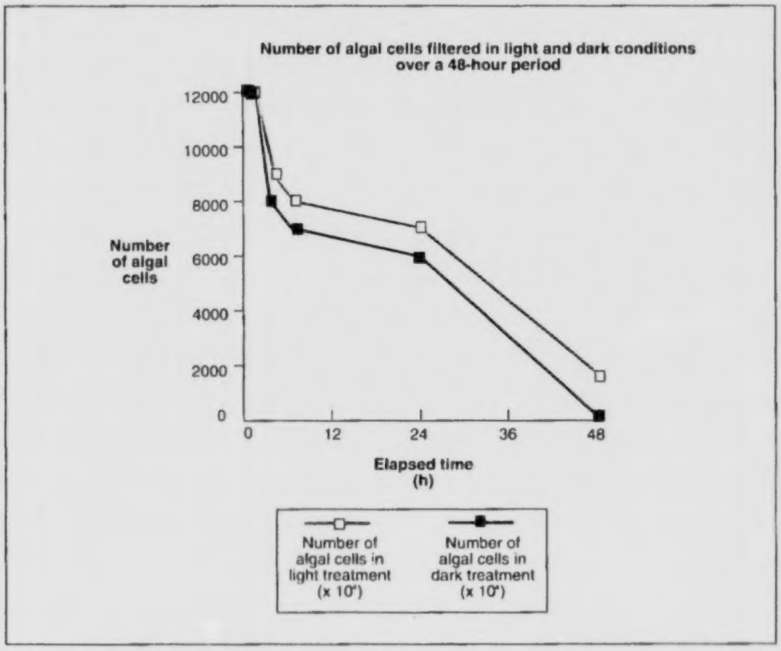
Phytoplankton, while abundant at the surface during the day, often sink to greater depths at night. Feeding at night may be more efficient than feeding during the day.

Sample Experimental Procedure

1. Set up 8 treatment aquaria with 1 L of water each. The water should be at the temperature of the maintenance aquarium.
2. Place 3 bivalves in each of the 6 aquaria.
3. One hour later, add 200 mL of algal culture to each treatment aquarium and take a sample from one to estimate the initial cell concentration.
4. Place 3 aquaria with bivalves and 1 control aquarium without bivalves in the light. Place the other 3 treatment and 1 control aquaria in the dark.
5. After 30 minutes, sample approximately 1 mL from each aquarium and estimate the cell density with a hemocytometer.
6. Repeat Step 5 at 2, 4, 6, 24, and 48 hours.
7. Correct the density values for any algal growth or disappearance unrelated to the presence of bivalves with data from the light and dark control aquaria maintained without bivalves.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph F. The number of algal cells present after incubating bivalves in aquaria in light and dark. These values are corrected for the controls.

TEST QUESTION

Why was it necessary to keep an aquarium in the light and one in the dark with algae, but without bivalves.

Interpretation

If the line for the number of algal cells in the dark treatment has a steeper negative slope than the line representing the number of algal cells in the light treatment as determined by a linear regression statistical test, these data support the hypothesis.

Answer to Test Question

In the light, it is likely that the algal population would grow. It was necessary to correct the counts for the possible increase in the number of algae in the light.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Use a light sensor to find changes in cultures due to algal growth. For more accurate measurement of single-cell green algae growth, use a spectrophotometer.

VARIATION 5

The Effect of pH on Food Uptake

Note to Teachers: In addition to the information found in Variation 1, the following material has been provided for Variation 5.

SYNOPSIS

Students will compare biodeposit production of oysters exposed to vinegar with those not exposed to vinegar.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- 10 mL undiluted 5.0% distilled vinegar
- pH test papers or pH meter (optional)

HYPOTHESIS GENERATION

Question

Is biodeposit production affected by acidic conditions?

Sample Hypothesis

The production of biodeposits will decrease if bivalves are exposed to acidic conditions.

Rationale

Bivalves respond to adverse environmental conditions, such as poor water quality, by closing the valves of their shells. When bivalves sense a reduction in pH, they respond by closing their valves and consequently their feeding activity also declines.

Sample Experimental Procedure

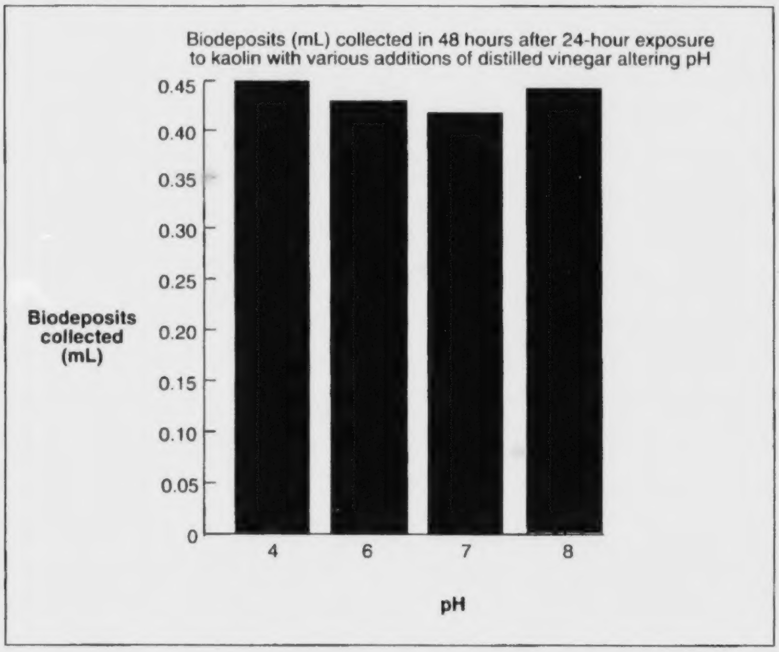
1. Do not add vinegar to 1 aquarium; it will be the control.
2. Amend each of 3 other treatment aquaria with different concentrations of vinegar to obtain pH values from 4 to 7.
3. Incubate and collect data as in Variation 1.

TEACHING TIPS

- Acid rain or other sources of low pH, such as mine effluents, can adversely affect freshwater bivalve populations by eroding shells (McMahon, 1991).
- Low pH decreases the availability of calcium.
- Use a pH meter or pH test papers to determine the acidity of the treatment aquaria.
- For *Crassostrea virginica*, lowering the pH slightly from the normal 7.75 produced a transient increase in pumping rate and later a decrease below the normal rate. At pH 6.5, activity always was reduced. At pH 4.25, it was reduced to 10% of the rate measured at a normal pH (Loosanoff & Tommers, 1947 in Jørgensen, 1966).
- Most freshwater bivalves prefer water with pH greater than 7. Some species are relatively insensitive to pH and can be found at a pH of 4.7 to 8.3 (McMahon, 1991).

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph G. Amount of biodeposits (mL) collected in 48 hours after 24-hour exposure to kaolin with various additions of distilled vinegar.

TEST QUESTION

Based on the results of your experiment, do you expect to find a reduction in the number of bivalves in an estuary receiving acidic industrial pollution compared with an estuary not receiving this form of pollution? Why or why not?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide electrical pH meters with module that speaks accurately and directly to the student. With current models, the larger the dial on the pH meter, the more accurately a light sensor can detect the location of the needle on the pH meter.

VARIATION 6

The Effects of Temperature on the Ciliary Action of Bivalves

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will measure the ciliary action of bivalves exposed to different temperatures.

Interpretation

If there is no difference in the total deposits collected in each treatment, reject the hypothesis. Here, there is a nearly complete overlap in individual values between the treatments, and there is no pattern in the production of biodeposits. These bivalves are not sensitive to a pH change in the aquarium water.

Answer to Test Question

One would not expect to find differences in the number of bivalves between undisturbed estuaries and those polluted with acid because no effect of the acid amendments was detected in the bivalves. Students may wish to re-evaluate their answer after learning what the pH created by the acid pollution was and what it was in the experiment.

TEACHING TIP

Only a small amount of carmine powder is needed. Dip the small end of the toothpick in the powder and tap off the excess.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- 1 dissecting microscope
- 1 screwdriver
- 1 pair heavy gloves
- 1 metal kitchen knife or scalpel
- carmine powder
- 1 stopwatch
- 1 metric ruler
- 1 flat toothpick
- 1 dissecting tray

SAFETY PROCEDURES

It is highly recommended that only the instructor open the mollusk shells and use the following precautions:

Wear a heavy glove and use a screwdriver in a direction away from the body to pry open the hinge of the oyster. Other species of bivalves are easier to open.

Exercise extreme caution when using the knife or scalpel. Cut in a direction away from the body. An accident is less likely to happen if the scalpel is very sharp and can cut the mollusk muscle easily. A dull scalpel may require force to open the shell.

HYPOTHESIS GENERATION

Question

What effect will temperature have upon the ciliary action of bivalves?

Sample Hypothesis

The higher the temperature is, the faster the ciliary action of bivalves.

Rationale

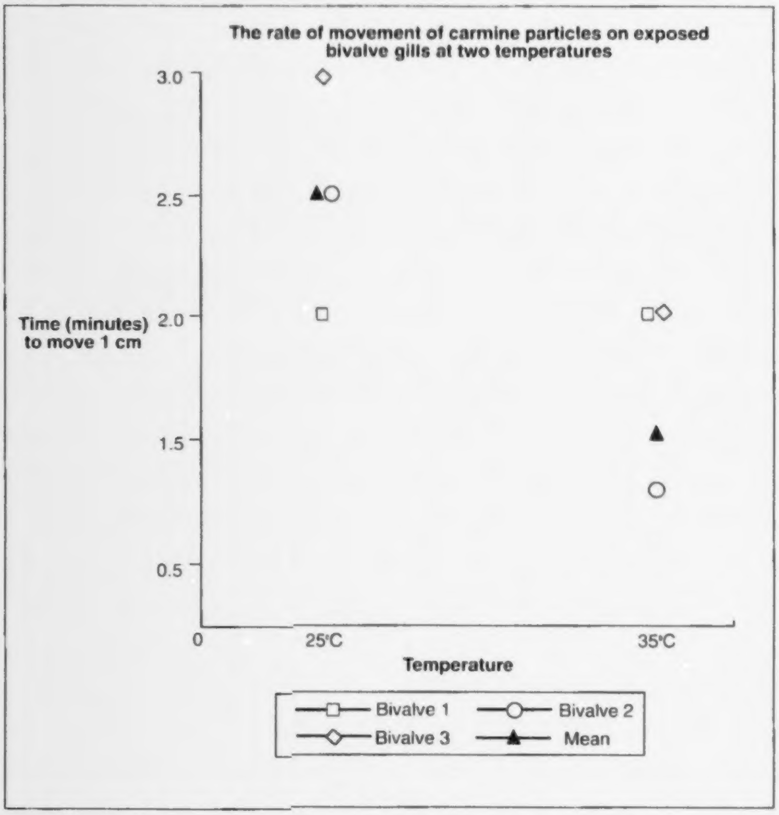
Metabolic rates are usually higher at higher temperatures in cold-blooded or ectothermic animals, such as mollusks. It seems likely that oysters will process particles faster. Faster processing would make more food available to support their higher metabolic rate.

Sample Experimental Procedure

1. Set up live bivalves in aquaria held at different temperatures. Allow them to acclimate 10 minutes before cutting open the valves.
2. Measure the effects of temperature on bivalve ciliary action in this way:
   a. Carefully separate the 2 halves of the shell that your instructor has opened previously.
   b. Immerse the bivalve in water at the same temperature as the aquarium from which it was taken.
   c. Use a dissecting microscope to focus on the gills.
   d. Apply the carmine powder by touching the water above the gill surface with the toothpick. See Figure 1. Watch the movement of the darkly colored particles along the surface of the gills. You will notice several particles making a streak. Time one end of the streak as it moves across the gills.
   e. Measure the distance the particle moved by putting a metric ruler on the microscope stage.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph H. Relationship of temperature to ciliary action in bivalves showing faster rates of particle movement at higher temperature.

TEST QUESTION

If you had maintained the bivalves at a temperature of 10°C for a period of several weeks, how would you expect the results to differ? Why?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have students who are visually impaired act as reporters and take temperatures with a Talking Thermometer.
- Have students who are visually impaired devise a classification key of mollusks if a collection of shells is available. There are directions in most biology texts for making a key.

Interpretation

If these data overlap, reject the hypothesis. If it took longer to move the same distance at the cooler temperature, accept the hypothesis. If it took longer to move that same distance at the warmer temperature, reject the hypothesis.

Answer to Test Question

If the bivalves initially were acclimated to the cooler temperature, one would expect them to move the carmine powder faster at that temperature than was measured in Graph H. When bivalves are held at a certain temperature for a period of weeks, they undergo biochemical adaptations that enable them to function optimally at that particular temperature. Also, this stress will be a general slowing of metabolic processes until the acclimation is complete.

VARIATION 7

The Effects of Salinity Level on the Ciliary Action of Bivalves

Note to Teachers: In addition to the information found in Variation 6, the following material has been provided for Variation 7.

SYNOPSIS

Students will measure the ciliary action of bivalves exposed to different levels of water salinity.

HYPOTHESIS GENERATION

Question
Will salinity affect ciliary action in bivalves?

Sample Hypothesis
The ciliary action of bivalves will not vary within a range of salinities.

Rationale
Living in an estuarine environment requires the ability to adapt to changing conditions of salinity.

Sample Experimental Procedure

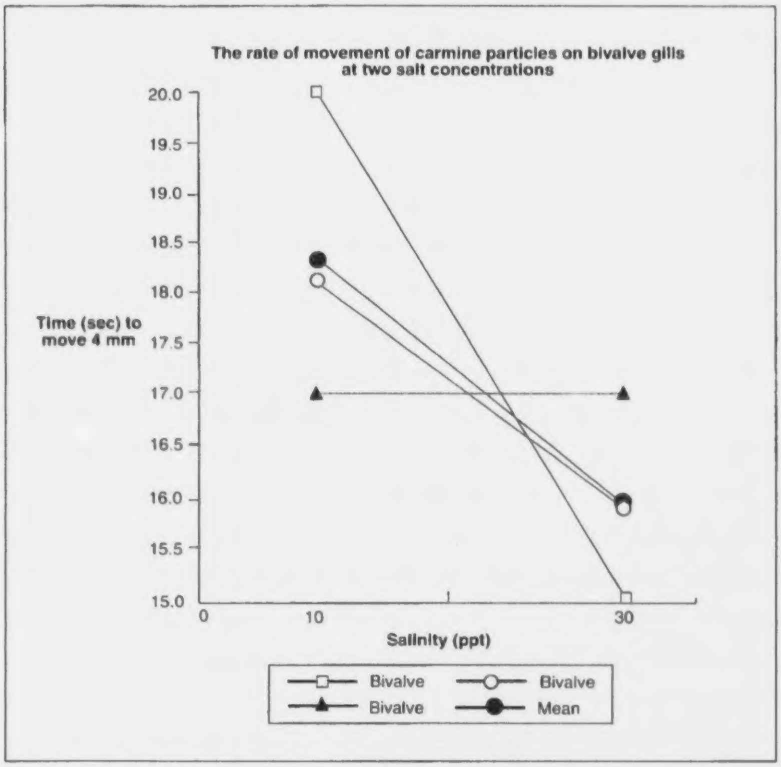
1. Set up bivalves in aquaria held at 2 different salinities with one at the same salinity as the maintenance aquarium.
2. Proceed with Step 2 in Variation 6 measuring the effects of salinity and not temperature on ciliary action.

TEACHING TIPS

- For the oyster, *Crassostrea virginica*, salinities from 25 to 39 ppt resulted in normal pumping activity; 20 ppt resulted in slower pumping; and below 13 ppt resulted in no pumping (Jorgensen, 1966).
- Use artificial seawater salts rather than sodium chloride, because ion imbalances with sodium chloride may result in the death of animals (Dietz et al., 1996).
- Provide background on estuarine communities if students have not observed this community previously.

DATA ANALYSIS AND INTERPRETATION

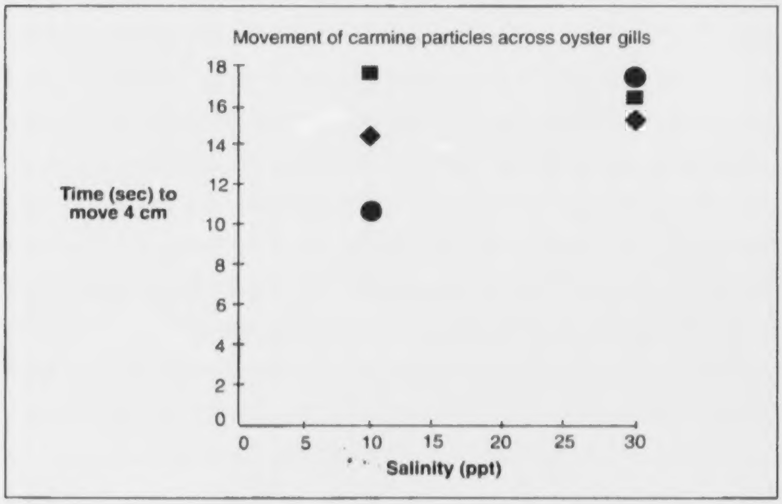
Sample Data



Graph I. Relationship of ciliary action and salinity concentration showing faster particle transport at higher salinities.

TEST QUESTION

If you obtained results like those shown in Graph J, how would you interpret them?



Graph J. The movement of carmine particles over bivalve gills at 2 salt concentrations.

Interpretation

If these data overlap, accept the hypothesis. If it took longer to move the same distance at the lower salinity, reject the hypothesis. If it took longer to move that same distance at the higher salinity, reject the hypothesis.

Answer to Test Question

There is more variability in the rate of particle movement at the lower salinity. There is no difference in the rate of movement at the high and low salinities because these data overlap completely. These oysters are not sensitive to the differences in salinity that were tested.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 6.

TEACHING TIP

Copper sulfate concentrations greater than 0.1 g/L are likely to be lethal after several days.

VARIATION 8

The Effect of Heavy Metal on Bivalve Biodeposit Production

Note to Teachers: In addition to the information found in Variation 6, the following material has been provided for Variation 8.

SYNOPSIS

Students will measure and compare the biodeposits produced by bivalves exposed to a heavy metal with those not exposed.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- $CuSO_4 \bullet 5H_2O$

DIRECTIONS FOR SETTING UP THE LAB

Add all amendments to the treatment aquaria before adding the bivalves.

SAFETY PROCEDURES

Cupric sulfate is a skin and respiratory irritant, and is toxic by ingestion and inhalation. It is strongly recommended that $CuSO_4$ solutions be purchased and that $CuSO_4$ powder not be used. If the powdered form is used to make solutions, the teacher should be the only individual who works with $CuSO_4$. Wear a mask, gloves, and lab apron.

Students should avoid contact with $CuSO_4$ solutions.

Wash hands after using the solutions.

Do not ingest the solutions.

Wear gloves and a lab apron.

Check your local safety guidelines for additional information about the safe use of $CuSO_4$, including storage and disposal.

HYPOTHESIS GENERATION

Question

Do heavy metals affect the filtering rate of bivalves?

Sample Hypothesis

The filtering rate of oysters exposed to a heavy metal will decrease as the concentration of the heavy metal increases.

Rationale

Heavy metals in large concentrations are toxic to bivalves.

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Sample Experimental Procedure

1. Use the kaolin concentration when the highest rate of biodeposit production in the linear part of the curve in all treatment aquaria was observed. Hypothetical data for the Core Experiment would be 20 mg/L.
2. Set up 4 treatment aquaria as follows:

Aquarium 1	Control with no cupric sulfate
Aquarium 2	0.001 g/L
Aquarium 3	0.01 g/L
Aquarium 4	0.1 g/L

3. Incubate and collect data as in the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Amount of biodeposits produced by asiatic clams exposed to various amounts of cupric sulfate.

	Amount of Copper Amendment (g/L)			
	0	0.001	0.01	0.1
Organism 1	0.17	0.02	died	0
Organism 2	0.15	Died	0	died
Organism 3	0.16	Died	0	0
Mean	0.16	<0.01	0	0

Create a scatter diagram of these data. Indicate the deaths on the graph.

TEST QUESTION

If copper or other heavy metals are so toxic that they cause bivalve death, how can they be used as indicators of contamination?

Interpretation

If fewer biodeposits are collected with high copper accept the hypothesis. If the amount does not change or increases, reject the hypothesis. Copper is very toxic. Organisms may die even in the least concentrated copper amendments. Clearly, filtration rate is less with high copper concentration. The sample size is so small that no significance can be attached to the death of organisms in the least concentrated amendment compared to the higher amendments.

Answer to Test Question

Even when bivalves die, they can be used as indicators of past contamination because their shells remain as an historical record of the population. Copper can accumulate in the shell of the animals as it lays down new shell throughout the growing season.

VARIATION 9

The Effect of Temperature on Bivalve Heart Rate

Note to Teachers: In addition to the information found in Variation 6, the following material has been provided for Variation 9.

SYNOPSIS

Students will measure the heart rate of bivalves exposed to different temperatures.

TEACHING TIPS

- There are no respiratory pigments in the hemolymph of most marine and freshwater bivalves. Their oxygen is dissolved directly in the hemolymph fluid, so their oxygen carrying capacity is the same as the water (McMahon, 1991).
- See Variation 6 for instructions on how to open bivalves.
- The pericardial cavity is equivalent to the coelom. In oysters, the heart is located immediately anterior to the adductor muscle. See Figure 1. The intestine travels the length of the cavity. The ventricle of the heart surrounds the alimentary tube in this cavity in most bivalves, but not oysters. If you dissect away the heart, you will reveal the digestive cavity. Refer to Figure 9 to locate the heart.
- All reports of molluscan heart rates show the beat is slowed by cooling and increased by heating within physiological ranges (Hill & Welsh, 1966).
- If the heart is large enough, you can see the beating without a microscope.
- The vast majority of bivalves do not have respiratory pigments. Their blood, a colorless fluid, is called hemolymph.

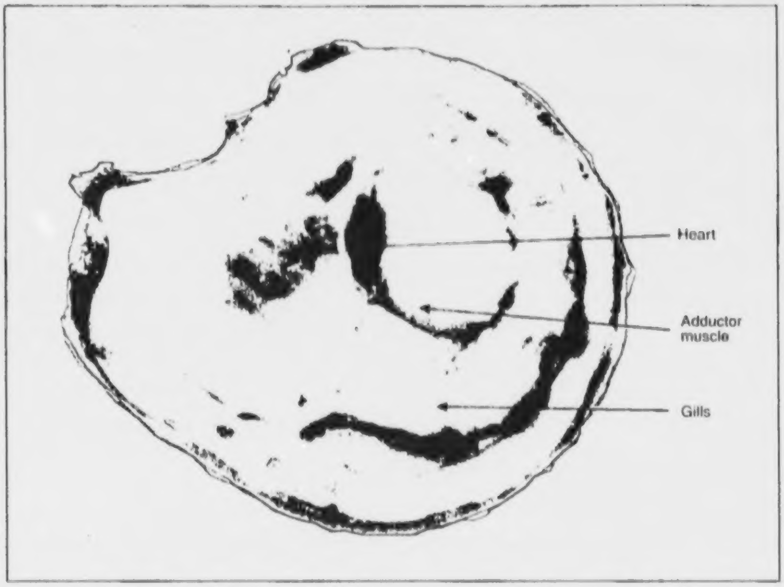


Figure 9. Cutting into the pericardial cavity just anterior to the posterior adductor muscle reveals the heart.

SAFETY PROCEDURES

See Variation 6 for instructions on how to open bivalves.

HYPOTHESIS GENERATION

Question

How does temperature affect the heart rate of bivalves?

Sample Hypothesis

The higher the temperature is, the faster the heart rate of bivalves.

Rationale

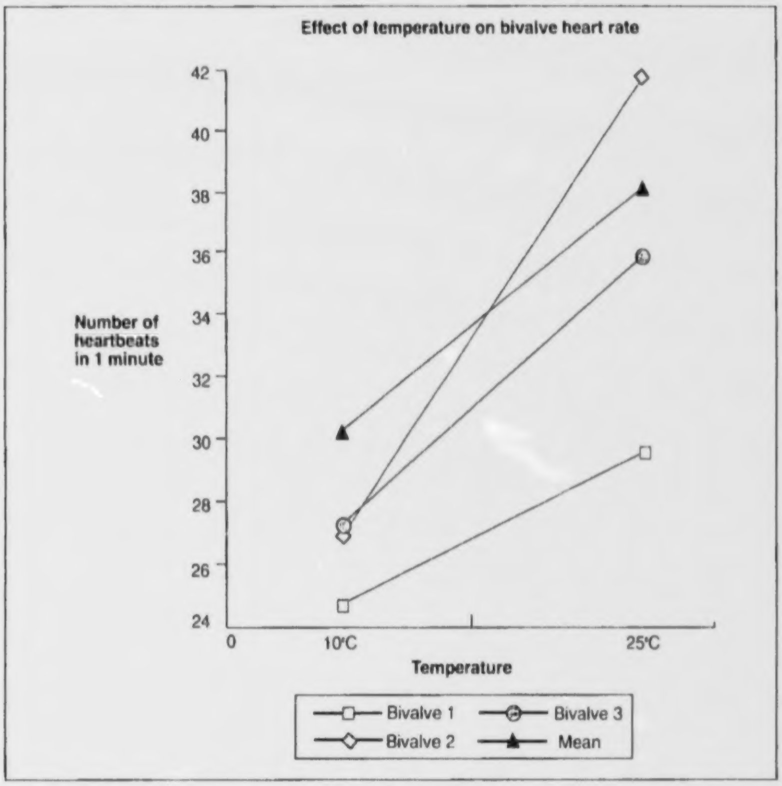
Higher temperature usually means greater metabolic rate and this should require more rapid circulation.

Sample Experimental Procedure

1. Set up bivalves in aquaria held at 2 different temperatures as in Variation 6. One aquarium should be the same temperature as the maintenance aquarium.
2. Measure the effects of temperature on bivalve heart rate in this way:
    a. Carefully separate the 2 halves of the shell that your instructor has opened previously. See Variation 6 for instructions on how to open bivalves.
    b. Immerse the bivalve in water at the same temperature as the aquarium from which it was taken.
    c. Cut the pericardium to see the heart beating.
    d. Using a dissecting microscope, locate the heart. If you have a medium-to-large size bivalve, you should be able to count the heart pulses without the microscope.
    e. Time and record the heart rate in 1 minute for each bivalve. Repeat the count 2 more times for each bivalve, and average the 3 numbers.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph K. Effect of temperature on bivalve heart rate.

TEST QUESTION

If you had maintained the bivalves at a temperature of 10°C rather than at 25°C, how would you expect the results to differ? Why do you expect this difference?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide students who are visually impaired with large oysters so they will be able to count the heart beat without the use of a microscope. A raised-line drawing of the cross section of the heart plus graphs of heartbeats should be included with this investigation.
- Record temperatures with a Talking Thermometer.

Interpretation

If these data overlap, reject the hypothesis. If there were fewer heartbeats at the lower temperature, accept the hypothesis. These data were quite variable at the higher temperature, but there was very little overlap. If there were more heartbeats at the higher temperature, the hypothesis can be accepted.

Answer to Test Question

If the bivalves were maintained at the cooler temperature, one would have expected them to have a lower heartbeat at the cooler temperature. Until they fully acclimate, all metabolic processes will be slow.

The Impact of Mollusks on Water Quality

Directions for Students

INTRODUCTION

FLAMING RIVER! The headlines of mid-1969 proclaimed. Indeed, pollution had created a combustible mess of the Cuyahoga River, a tributary to Lake Erie. All it took to ignite the river was a spark from a steel-mill rail car to create the absurd situation that alerted many complacent Americans to the damage we had done to our waterways. The first Earth Day was celebrated the following year, and we were made aware of extreme pollution in many waterways. Lake Erie looked like pea soup (Luoma, 1996). The Chesapeake Bay where oysters were once so abundant that they filtered the entire volume of the bay in just a few days, now had so few oysters that it required a year to filter the same volume of water (Newell, 1988). Filter-feeding mollusks are among the losses attributed to pollution. These creatures and many other endangered aquatic organisms are less charismatic than the familiar endangered wolves or otters. In Illinois alone, 67% of all state-listed endangered animals and 22% of all threatened and endangered species are aquatic (Cummings & Mayer, 1996).

Clams, oysters, and mussels can be forms of natural pollution, too. See Figure 1 for representative mollusks.

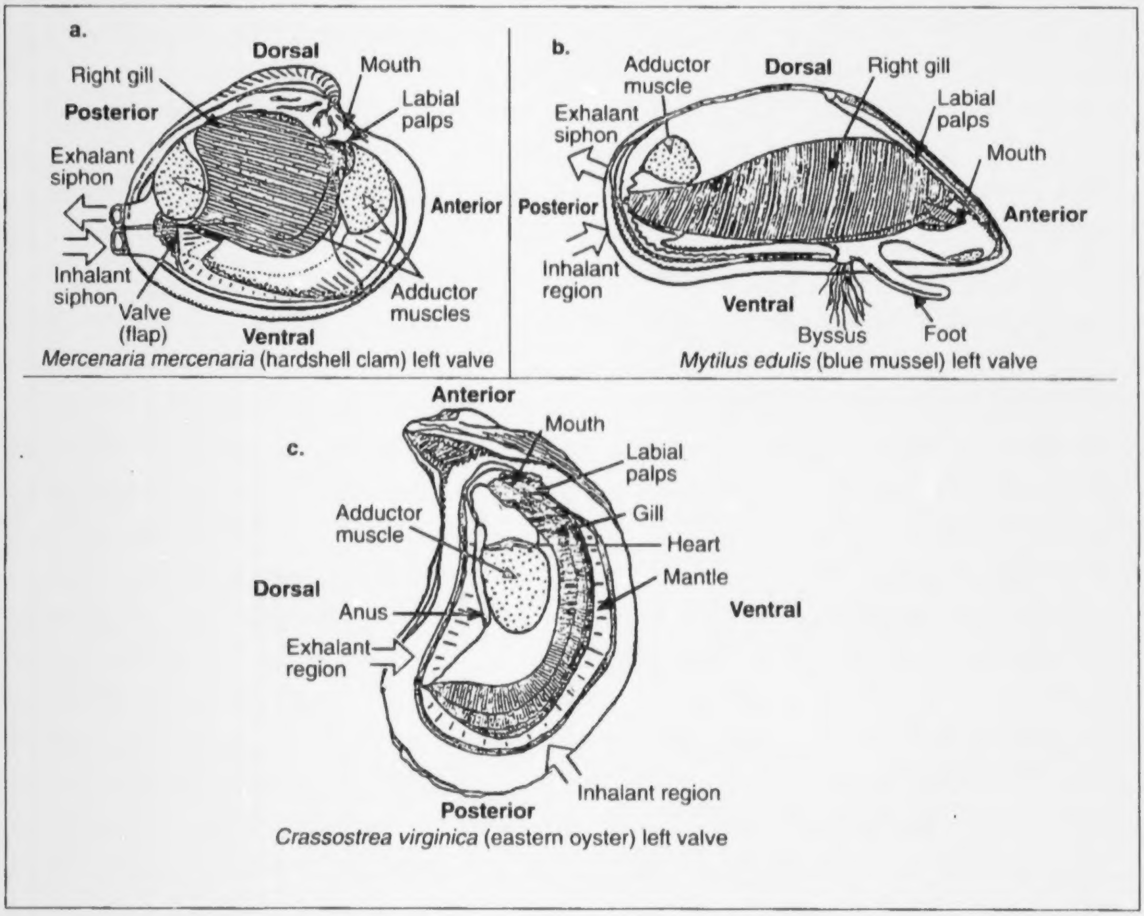

a.
Dorsal
Right gill
Mouth
Labial palps
Posterior
Exhalant siphon
Anterior
Inhalant siphon
Valve (flap)
Adductor muscles
Ventral
Mercenaria mercenaria (hardshell clam) left valve
b.
Adductor muscle
Dorsal
Right gill
Labial palps
Exhalant siphon
Mouth
Posterior
Anterior
Inhalant region
Ventral
Byssus
Foot
Mytilus edulis (blue mussel) left valve
Anterior
Mouth
c.
Labial palps
Gill
Adductor muscle
Heart
Mantle
Dorsal
Ventral
Anus
Exhalant region
Inhalant region
Posterior
Crassostrea virginica (eastern oyster) left valve

Figure 1. a. *Mercenaria mercenaria* (hardshell clam). b. *Mytilus edulis* (blue mussel). c. *Crassostrea virginica* (eastern oyster).

For example in 1988, zebra mussels arrived in the Great Lakes probably in the ballast water of a ship from Europe. Since they reproduce so quickly and have few natural predators, they quickly clogged water-intake pipes, coated boat hulls, and contributed to extensive mortality or complete elimination of some native bivalves. By the end of 1995, 20 of the 38 states east of the Rocky Mountains had been invaded by zebra mussels (Ram & McMahon, 1996). The news about zebra mussels is not all bad. They have done such a terrific job of filtering that light now penetrates deep into Lake Erie and eight native species of plants have returned after an absence of 30 to 50 years (Anonymous, 1996).

When planktonic algae containing a powerful nerve-toxin are abundant, shellfish concentrate the poisons. They also accumulate other toxins including heavy metals, like copper and mercury, and bacterial pathogens from human sewage (Boyle, 1981).

In this exercise you will investigate some of the behavior of bivalves that makes them good news and bad news for the environment.

OBJECTIVES

At the end of this lab, you should be able to:

- Describe the ecological role of filter-feeding mollusks in fresh and/or saltwater ecosystems.
- Identify the major anatomical structures and functions of a filter-feeding mollusk.
- Describe how turbidity and salinity affect the behavior of a filter-feeding mollusk.
- Explain how a population of oysters, clams, or mussels impacts water quality.
- Maintain strict procedures needed to support the organisms' health and basic functions.

SAFETY NOTES

Wash your hands before and after the lab.

Do not pick up an aquarium containing water.

Carry expensive glass instruments like hemocytometers in unbreakable containers.

Do not discard mollusks or wash water into local waterways.

Do not eat any of these organisms.

Do not open any shellfish using a sharp instrument, such as a scalpel. Your instructor will do this.

Wear safety goggles and lab apron.

MATERIALS NEEDED

For each team of four students, you will need the following:

- 5 live freshwater clams, such as *Corbicula fluminea* or saltwater hardshell clams *Mercenaria mercenaria*, marine blue mussels *Mytilus edulis*; 5 live oysters, such as *Crassostrea virginica*
- 0.5g (3t) kaolin powder
- 3 3.8-L (1-gallon) aquaria or wide-mouthed glass or plastic jars
- 12 L artificial seawater
- 1 aerator
- 1 thermometer (°C)
- 15 petri dish halves
- 1 Pasteur pipette
- 10 10-mL graduated cylinders

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature or web search on the topic of filter-feeding bivalves and pollution. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

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HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. What makes this question an interesting or important topic for investigation?
3. Why is it important to control variables?
4. What is the variable in your experiment?
5. What will you measure?
6. How will you control other potential variables?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

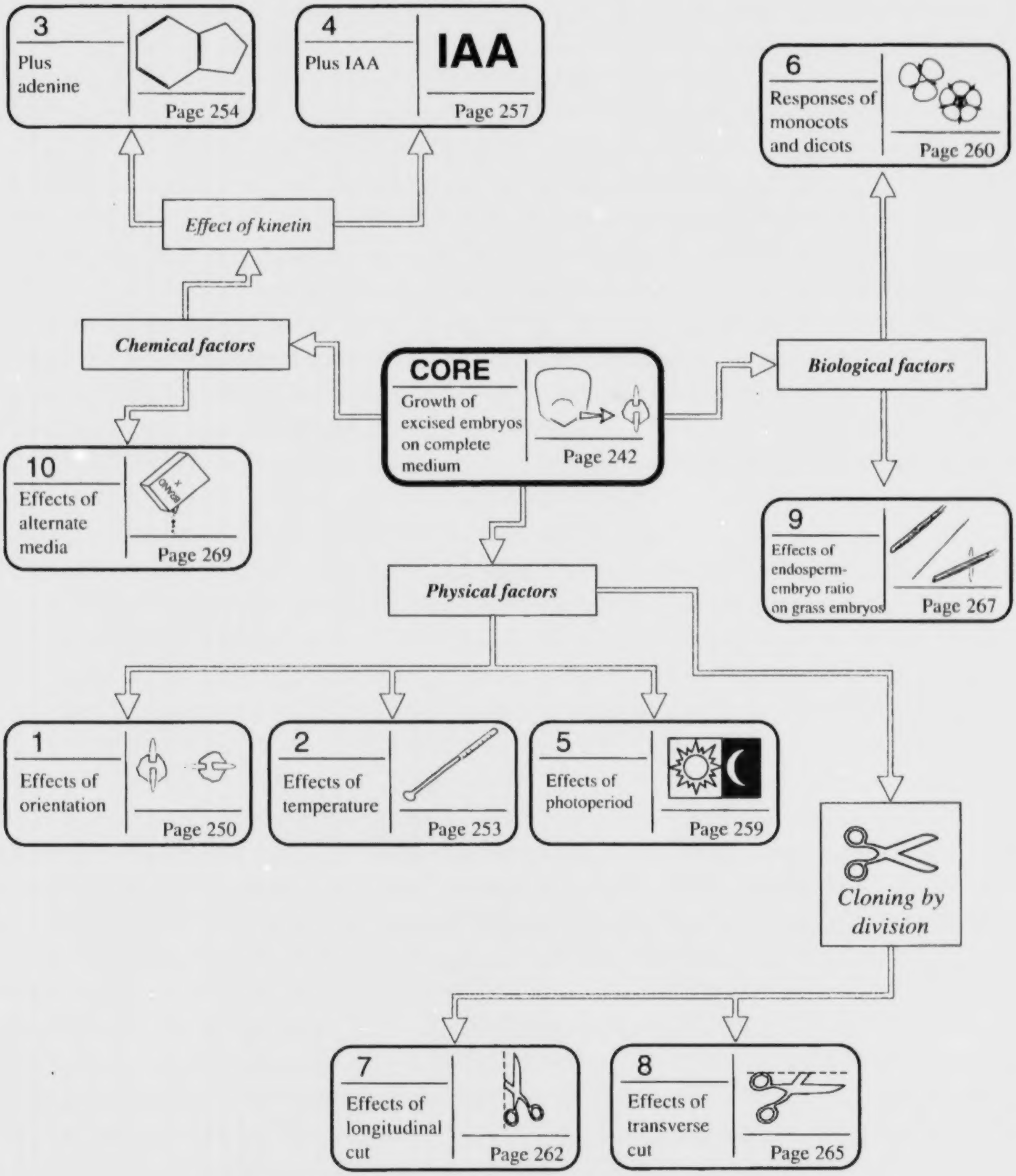
1. Construct a bar graph showing the total amount of biodeposits collected for bivalves in each treatment.
2. Do your data support or refute your hypothesis?
3. Using your specific data, explain your answer to Question 2.
4. Compare your data with that of other groups. Are these data the same or different? Why or why not?
5. Will the amount of biodeposits always be the same under the conditions you used?
6. What could cause differences? Be specific.

DESIGN OF VARIATIONS OF CORE EXPERIMENT

- How do filtering rates compare for different species of bivalves?
- How will temperature affect the production of biodeposits?
- Are oysters adaptable to a range of salinities?
- What effect does light have on the filtering rates of bivalves?
- Is biodeposit production affected by acidic conditions?
- What effect will temperature have upon the ciliary action of bivalves?
- Will salinity affect ciliary action in bivalves?
- Do heavy metals affect the filtering rate of bivalves?
- How does temperature affect the heart rate of bivalves?

Plant Tissue Culture

At-A-Glance Map of the Core Experiment and Its Variations


3
Plus adenine
Page 254
4
Plus IAA
IAA
Page 257
6
Responses of monocots and dicots
Page 260
Effect of kinetin
Chemical factors
CORE
Growth of excised embryos on complete medium
Page 242
Biological factors
10
Effects of alternate media
Page 269
9
Effects of endosperm-embryo ratio on grass embryos
Page 267
Physical factors
1
Effects of orientation
Page 250
2
Effects of temperature
Page 253
5
Effects of photoperiod
Page 259
Cloning by division
7
Effects of longitudinal cut
Page 262
8
Effects of transverse cut
Page 265

NOTES

Plant Tissue Culture: Embryo Isolation and Tissue Culture Initiation

SYNOPSIS FOR CORE EXPERIMENT

Students will excise the embryo of a plant seed to compare the growth of complete and partial embryos in vitro on a nutritionally complete medium. They will form hypotheses and design experiments to analyze the effect of various physical and/or chemical factors on the growth of the embryo.

APPROPRIATE BIOLOGY LEVEL

Advanced or highly motivated introductory level students.

TEACHER PARTNERS

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SCIENTIST PARTNER

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Directions for Teachers

Note: This investigation has been modified from the following sources, and is reprinted here with permission: Herbert, R. & Thompson, D.J. (1993). Culturing Plants from Embryonic Plant Tissue. Princeton, NJ: 1993 Woodrow Wilson Biology Institute.

Thank you to Paul Bottino (University of Maryland), Lisa Darmo (Carolina Biological), and Carol Reiss (Cornell University) for their assistance.

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 250. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Identify the stages of embryo development.
- Excise an embryo from a plant seed.
- Inoculate a sterile, nutritionally complete growth medium with the excised embryo.
- Observe and record the growth of a plant embryo in vitro.

MATERIALS NEEDED

For teacher preparation, you will need the following for a class of 24:

- 4.0 L sterile, distilled water
- 42.4 g M&S Basal Tissue Culture Medium with sucrose and agar (Sigma M9274) or prepared M&S medium (Carolina #195747)
- 1 hot plate with magnetic stirrer
- 80 15 x 100-mm sterile, disposable petri dishes
- 1 autoclave
- 1 L 10% household bleach solution
- 750 mL rubbing alcohol or 400 mL 95% ethanol
- 1 mL liquid dishwashing detergent with antibacterial agent
- 2 to 4 10-gallon aquaria

(continued on p. 240)

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Introduce the lab and practice excising embryos on a Monday or Tuesday.

Day 2 (45 minutes)

- Practice excising embryos using aseptic technique.

Day 3 (30 to 45 minutes)

- Inoculate cultures.

Days 4 to 8 (15 to 30 minutes)

- Observe and record data.

Day 9 (45 minutes)

- Analyze data and draw conclusions.

PREPARATION TIME REQUIRED

60 minutes

- Locate and/or gather glassware, fresh supermarket corn, and other equipment.

15 minutes

- Prepare the agar plates.

90 minutes

- Prepare the culture medium, dispensing medium into the culture vessels and autoclaving the vessels.

30 minutes

- Prepare solutions.

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TEACHING TIPS

Seeds

- If fresh supermarket corn is unavailable year-round in your area, you may want to schedule this laboratory for early fall rather than use dry corn. Alternatively, soak dry corn seeds for 24 to 48 hours before the laboratory.
- Dry seeds must be surface-sterilized by briefly soaking in a 10% bleach solution. The amount of time allowed for surface sterilization of the seeds will vary between 2 to 6 minutes, depending upon the thickness of the seed coat. For example, bean seeds will take less time than corn seeds. Then, soak for an appropriate amount of time to soften the seed coat. Corn should be soaked 36 to 48 hours; pole or lima beans, 3 to 4 hours.
- Lysol® is not recommended for use as a disinfectant on surfaces coming into contact with the embryo, as it can destroy the delicate tissues.
- Students need to practice recognizing and excising the embryo from the seed prior to the actual lab using this procedure.

Medium

- Sigma Chemical Company recommends that the entire contents of the plant growth medium bottle be used after opening due to the hygroscopic nature of the dry medium. However, with careful handling and storage of unused medium under refrigeration in an airtight container, dry medium may be kept for up to three months. Prepared and autoclaved medium will keep for a week under refrigeration. Medium may be microwaved to liquefy to pour additional plates.
- If petri dishes are used, pour the medium into plates in a transfer cabinet.
- If test tubes or other narrow-mouthed vessels are used, the surface area for explants may be increased by allowing the medium to solidify at a slant.
- If 1 L of medium or approximately 66 petri dishes is more than you need for the Core Experiment, consider autoclaving 15 mL aliquots in culture tubes. The agar can be melted later in a hot water bath, and students can pour their own individual plates. Or, autoclave the medium in 100-

(Continued on page 241)

(continued from p. 239)

- 2 to 4 60-cm x 90-cm sheets of heavy-weight, clear plastic
- 1 pair of scissors
- 1 roll of duct tape
- 1 artificial lighting system with 24-hour timer

You will need the following for each group of two to four students in a class of 24:

- 1 250-mL spray bottle of 70% ethanol or 10% household bleach
- 1 sterile petri dish with M&S Basal Medium with sucrose and agar
- 1 sterile petri dish with water agar
- 1 permanent marking pen
- 1 bottle of antibacterial soap
- 1 fresh supermarket ear of yellow corn (*Zea mays*) or dry field corn seeds
- 50 mL sterile water
- 25 mL 10% household bleach-soap solution
- 3 sterile, disposable petri dishes
- 1 sterile film canister or 1 250-mL beaker with aluminum foil cover
- 1 timer
- 1 shallow pan
- 1 sterile surgical scalpel, Bard-Parker No. 3 handle
- 1 sterile No. 10 blade
- 1 sterile No. 11 blade
- 2 sterile forceps, 11-cm fine point
- 1 500-mL beaker
- 2 sterile paper towels
- 2 2.5 x 15-mm Parafilm™ or Petri Seal™ strips

SAFETY PROCEDURES

Wear safety goggles and lab coats/aprons at all times.

Wear protective gloves when wiping surfaces with bleach or alcohol, and while mixing solutions.

Keep open containers of alcohol away from the same work area as a flame.

Scalpels/razor blades should be used in a direction away from the body when excising the embryo.

Dispose of sharp objects, such as razor blades or broken blades, in proper containers.

Determine if students are allergic to latex, if gloves are used.

Re-autoclave all medium to destroy possible pathogens introduced by accident during transfer of the embryo before cleaning glass petri dishes or disposing of plastic ones after completion of the experiment.

If you use flame sterilization, do not put the flame source inside the transfer cabinet. Heat may break the glass or discolor plastics.

Exercise extreme caution when wearing gloves to flame sterilize.

Do not place the flaming tools back into the alcohol jar to extinguish flames.

DIRECTIONS FOR SETTING UP THE LAB

Three to five days before

Prepare the tissue culture medium, dispense, and sterilize as described in the following section.

M&S Basal Medium with Sucrose and Agar

1. Stir 1 L of distilled water while adding 42.4 g powdered medium.
2. Heat until the solution is clear, stirring continuously. Do not boil!
3. Autoclave in a container no more than half-full for 15 minutes at 15 pounds per square inch (psi).
4. Allow the medium to cool slightly before pouring approximately 15 mL into each of 66 petri dishes.

Alternatively, if the medium is purchased already prepared, heat in hot water bath until melted.

Water Agar

1. Stir 200 L of distilled water while adding 1.6 g of agar.
2. Heat until the solution is clear, stirring continuously. Do not boil!
3. Autoclave in a container no more than half-full for 15 minutes at 15 pounds per square inch (psi).
4. Allow the medium to cool slightly before pouring approximately 15 mL into each of 66 petri dishes.

One day before

Disinfectant Solutions

10% household bleach (0.5% sodium hypochlorite*) solution
Combine 100 mL household bleach with 900 mL distilled water.

70% Ethanol

Use 70% rubbing alcohol or combine 370 mL 95% ethanol with 130 mL distilled water.

Surface Sterilization Solution

10% Household Bleach-soap (0.5% sodium hypochlorite*) Solution

1. Combine 100 mL household bleach with 900 mL distilled water.
2. Add 2 to 3 drops dishwashing liquid with antibacterial agent.

*(*Note: Commercially prepared bleach is usually a 5.25% sodium hypochlorite solution. The dilutions created by each solution above are given in parentheses.)*

TEACHER BACKGROUND

Content Information

Plant tissue culture generally is described as the aseptic, in vitro growth of any plant part on or in a nutrient medium. The medium may be solid or liquid, depending upon the application. The underlying concepts of the techniques initially were developed in the late 1800's and early 1900's. The German plant physiologist G. Haberlandt refined them in 1902.

In any plant tissue culture experiment:

- A plant section called the *explant* is removed from the original plant to eliminate any cell, tissue, and/or organ interactions of the intact plant.
- The explant is grown in a chemically defined and physically controlled environment.
- An aseptic environment is maintained to eliminate the effects of external plant pathogens on the development of the explant.

TEACHING TIPS

(continued from p. 240)

mL aliquots for later use.

- Depending upon the level of the students in the class, the teacher may wish to prepare the medium, dispense it into the tissue culture vessels, and autoclave the prepared vessels in advance. Alternatively, if measuring pH adjustment, dispensing of medium, and autoclaving are skills to be taught and reinforced, this may be done as a preparatory lab exercise. This will require an additional 45 minutes and additional time for autoclaving.
- Always use a container twice the size of the final volume of medium being prepared.
- Add the powdered medium while stirring the water.
- If possible, use double distilled water for the medium preparation.
- The medium can be adjusted to a specific pH by using dilute solutions of KOH or HCl.
- After the medium is prepared, pour the medium into the containers. Autoclave any nonsterile, glass containers with medium for 15 minutes at 15 pounds per square inch (psi). Be sure the containers can be autoclaved safely. TAKE CARE NOT TO "OVERCOOK".

Disinfectant Solutions

- Mix 1 to 2 L quantities of the ethanol and the household bleach solutions in advance to reduce student preparation time.

Transfer Chamber *(optional)*

- In the absence of a laminar flow hood for excision and implanting, minimize air flow in the work area by using one of the following techniques:
  - Turn an aquarium sideways, wash with warm soapy water, and then wipe down thoroughly with 10% bleach solution. The opening can be covered with a heavy-weight, clear plastic sheet that also has been cleaned and wiped with bleach solution.
  - Staple, sew, or tape together heavy-weight plastic over a hanging file folder support frame and wash with soapy water and bleach.

(continued on p. 242)

TEACHING TIPS

(continued from p. 241)

- Use a commercial transfer cabinet or build one from a sheet of shower paneling and a piece of Plexiglas™. The bottom can be open if you will be working on a counter that can be wiped clean. If you build cabinets, you may want to hinge the sides and set the clear cover on with a lip across the back to hold it in place. A cabinet made this way can be collapsed for storage.
- If the experiment must be performed on open lab benches, cardboard "walls" can be constructed to reduce air flow through the work area. This method is considerably less effective in preventing contamination than the previous ones.

- Wipe down the inside of the box after each use with 10% bleach solution if using one of the alternatives for a laminar hood.
- Clear, heavy-weight plastic may be purchased in a fabric store by the yard.

Culture Vessels

- Culture vessels should be wide-mouth and short enough in height to use standard (10-cm) forceps. Longer forceps are prohibitively expensive. Baby food jars with reclosable caps are an excellent alternative to expensive laboratory vessels.
- If narrow-mouthed vessels such as test tubes or culture tubes are used, allow the sterilized vessels to cool and the medium to solidify on a slant to increase surface area for embryo implants.

Sterile Technique

- Sterile procedures are a "must" and include sterilizing:
  - the work area with a 10% bleach solution.
  - instruments by autoclaving in autoclave paper or soaking for 20 minutes in a covered container with 70% ethanol.
- Sterile plastic or glass petri dishes should be used for storing and manipulating the seeds.

(continued on p. 243)

There have been many practical applications and extensions of Haberlandt's original work. However, he was not successful particularly at growing explants on nutrient media, as it did not contain auxins, critical plant growth factors. Auxins were unknown at the turn of the century. More recent experiments have used improved methods of plant tissue culture as a way to:

- Maintain pure genetic traits in desirable plants by cloning, the technique of growing whole plants from plant sections.
- Insure development of the embryo in cases where the endosperm inhibits proper growth when the seed is planted in soil. This is true in several plant species that have evolved seeds which germinate only after environmental cues, such as fire, flooding, light exposure, or cold weather.
- Achieve faster growth of the embryo than if it were germinated in soil.

Plants used in tissue culture experiments are selected on the basis of the traits that are desirable to maintain and propagate. Seeds used for embryo studies tend to be readily available and have embryos that are easy to remove. For this introductory exercise in embryo excision, corn and/or bean seeds will be used as they are the easiest to manipulate.

Some facts about tissue culture that may be of interest to students are:

- Whole plants can regenerate from a single, undifferentiated cell.
- Researchers can manipulate explant tissue to produce a genetically altered plant using molecular biology, technology and genetic engineering.
- Plant tissue culture is not confined strictly to research labs in universities, but is an important tool used in agriculture and industry.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

Correct Concept	Misconception
• A seed is composed of differentiated tissue. Only the embryo develops into a new plant.	• The entire seed grows into a plant.
• Cotyledons are immature leaves of the plant embryo. The cotyledons of dicots, such as bean, no longer contain endosperm but still serve as food reserves. In monocots such as corn, endosperm is still present but separate from the cotyledons.	• Both monocot and dicot cotyledons are immature leaves of the embryo that contain endosperm.
• Seeds may be germinated in vitro and embryos grown on chemically defined media.	• Seeds must be planted in soil in order to grow.
• Specific plant hormones promote differential growth.	• Fertilizers are the only chemicals that promote plant growth.
• Seeds are living.	• Seeds are nonliving.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

This experiment is a natural extension of a study of plant structure and function. Possible ways to introduce the lab include:

- Introduce the topic of plant tissue culture using the information from the Teacher Background section by demonstrating the techniques of embryo excision and transfer of the excised embryo into the culture vessel. A video camera setup or overhead projection transparencies and/or blackboard diagrams may be used to instruct an entire class in the method of embryo excision. Then, have individual students practice excising the embryos.

- Relate the lab to current topics in genetic engineering. Some questions you might ask are:
  - Cloning animals has been in headline news since the 1980's. Can an animal or a plant be cloned from a single cell to maturity? Guide student discussion of this topic with the following information: A plant can be cloned from a single nucleus of a mature cell. In animals, mammals usually cannot be cloned to maturity from a single cell with the exception of gametic cells. Ask students to research the original paper describing the sheep Dolly's cloning.
  - If so, what are the advantages of cloning plants as compared with propagating them from seeds?
- Demonstrate the concept that each plant cell in a differentiated organism has all the genetic information for the production of a complete, mature organism. Some possible demonstrations include showing:
  - The growth of a potato plant from a potato "eye."
  - The *Kalanchoe daigremontiana* (maternity plant) with plantlets growing from notches in the leaves. Ask students to compare the plantlets with the parent plant and to speculate on what will happen if the plantlets are removed and planted separately in soil.
- Show students a corn embryo already growing in a nutritionally complete medium. Explain that the embryo was removed from its natural food source before it was placed on this medium. Ask students to:
  - Use their knowledge of the nutritional requirements of a growing plant to predict the components of the growth medium.
  - Predict how the plant would grow if a particular growth ingredient were left out of the medium, or a new ingredient, such as a hormone, were added to the medium.
- Place two geranium plants side by side at each lab station. One should be grown using tissue culture (e.g. an infertile hybrid variety), and the other should be grown from seed. Have students observe the two plants and record their observations. Then, tell them that the method used to reproduce the two plants is different. Ask them to use their list of observations to determine the method of reproduction for each plant.
- Introduce students to the influences of hormones on plants using one of the following demonstrations:
  - Show a plant that has grown after having the terminal bud removed and one that has not had the bud removed.
  - Show a cutting from a plant that was treated with a commercial rooting medium and one started at the same time but without the treatment.
- Assign students to search the Internet for three sites on the World Wide Web (www) that contain information on plant tissue culture.

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses. Generate a discussion about plant tissue culture using one or more of the following questions:

- Why fool with Mother Nature? Haven't plants been reproducing successfully on their own for millions of years?
- What are some ways that plant tissue culture could be used in space or in the biosphere?
- What nutritional and environmental conditions are required for plant growth in vitro?

TEACHING TIPS

(continued from p. 242)

- Minimize contamination by stressing that students wash their hands thoroughly with an anti-bacterial soap.
- Use only sterile instruments to hold or cut seeds and to transfer embryos to culture vessels.
- Wearing sterile gloves may be necessary for students who are allergic to disinfectant solutions, or when steadying seeds for embryo removal.
- Students should practice excising embryos in petri dishes. A supervising partner can watch for asepsis.
- Sterilize the medium in the culture vessels that will be used for the experiment.
- Sterilize water and glass petri dishes unless disposable plastic dishes are being used.
- The surface of the Parafilm™ that is next to the paper is sterile. If sealable plastic bags are used, the inside of the bag is sterile.
- If your students will flame sterilize tools, demonstrate holding the tools with the tips down so that burning alcohol does not flow onto their hands.
- Data analysis can be done outside of class. Most of the manipulations are simple math or can be done on personal calculators.
- Schedule lab setup so that weekend data collection is not necessary.

TEACHER'S NOTES

- Can a complete plant grow from a partial embryo?
- How is plant tissue culture used in research?
- How can a plant in tissue culture be protected from pathogens?
- What are some similarities and differences between plant tissue culture and aqua culture?

Sample Hypotheses

- If an embryo is cut longitudinally and cultured on nutritionally complete medium, then each half will develop into a normal plant.
- If an embryo is cut transversely, then one half will develop only as a shoot and the other half will develop only as a root.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 249.

Question

Is the embryo of a seed capable of growing without endosperm?

Core Hypothesis

If a corn embryo is excised from a seed and transferred to a nutritionally complete growth medium, then it will produce shoot and root growth comparable to an embryo excised with its endosperm intact.

Rationale

The culture container should provide the protection offered by the seed coat. If the medium is nutritionally complete, it should substitute for the endosperm.

Procedure

Work Area

1. Wipe the laboratory work bench and transfer chamber with 10% soap-bleach solution. (Note: Some of the following procedures were originally described by Green and Phillips, 1975.)
2. Allow the surfaces to air dry.
3. Obtain 1 sterile petri dish with M&S Basal Medium. Label it "without endosperm."
4. Obtain 1 sterile petri dish with water agar. Label it "with endosperm."

Excision of Corn Seeds and Embryo Extraction

1. Cut 3 to 4 corn kernels from a fresh, mature corn cob with a scalpel.
2. Make a small incision beneath 1 kernel very close to the cob in the row adjacent to where the kernels were removed in Step 1. Be careful not to cut the embryo. See Figure 1.

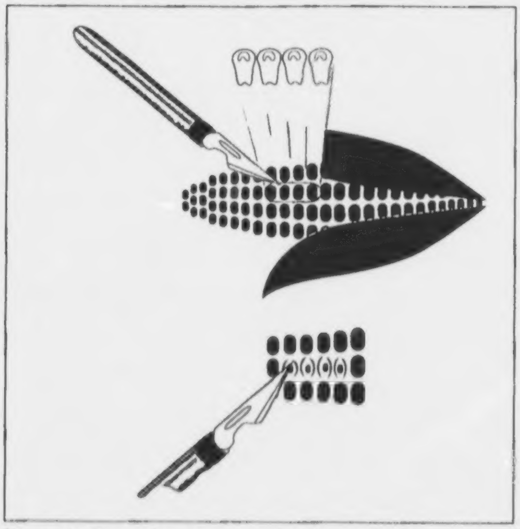

Figure 1. Removing a corn kernel from a fresh, mature cob.

3. Carefully slit the crown of the corn kernel with a scalpel.
4. Gently squeeze the sides of the embryo between the thumb and index finger. The embryo should pop out. It is a cream-colored, oval-shaped hard tissue found in the softer endosperm tissue, approximately 1 to 2 mm in length. See Figure 2.

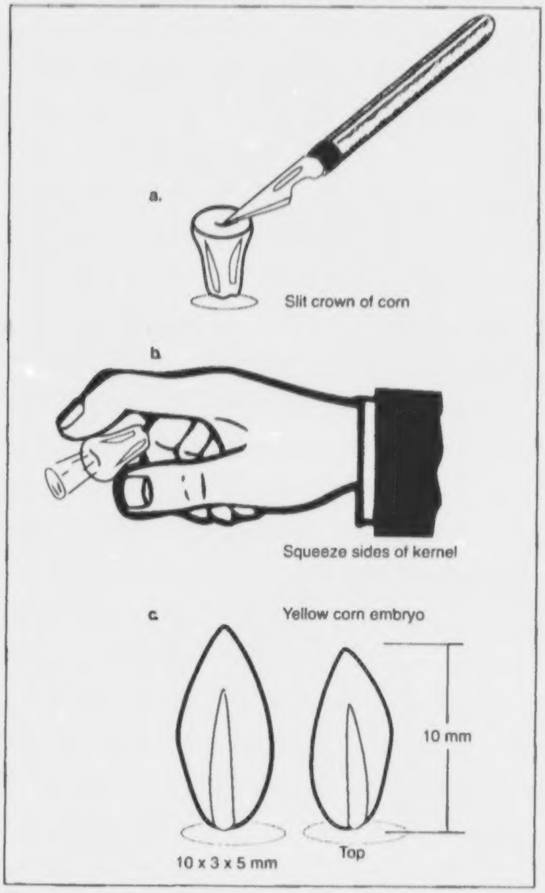
a.
Slit crown of corn
b.
Squeeze sides of kernel
c.
Yellow corn embryo
10 mm
10 x 3 x 5 mm
Top

Figure 2. Excising the embryo from a fresh corn kernel.

TEACHER'S NOTES

TEACHER'S NOTES

5. Lift up the top half of the petri dish containing the medium just enough to place the embryo directly on the medium while keeping the top half of the dish over the medium. Replace the top half of the dish immediately after placing the embryo.
6. After the first kernel is removed from the cob intact, additional kernels may be removed by simply wriggling them slightly with your fingers. See Figure 3.

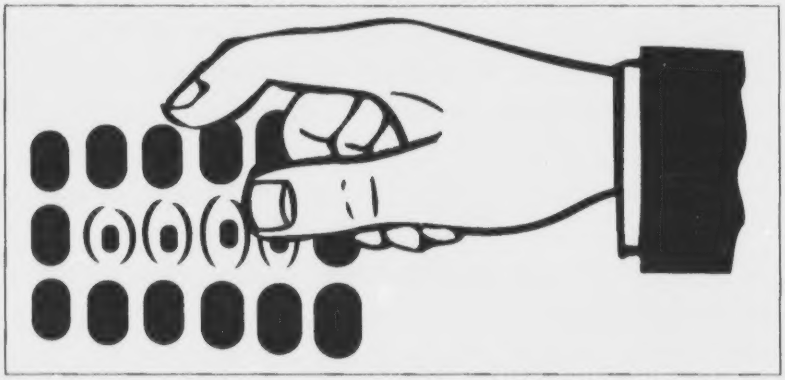

Figure 3. Removing additional kernels with fingers.

7. Repeat Steps 3 to 5 to obtain 4 additional embryos.

Surface Sterilization if Dry, Not Fresh Seeds Are Used *(optional)*

1. After soaking the seeds in sterile water for 2 days, place 5 corn kernels into a film canister containing 70% ethanol or a 250-mL beaker containing 100 mL of 10% bleach solution.
2. Cover the container and gently swirl for 10 minutes.
3. Pour the bleach solution into a 500-mL beaker.
4. Rinse the kernels 3 times in 50 mL of sterile, distilled water. Use aseptic techniques. Pour the rinse water into the 500-mL beaker.

Incubation and Observation

1. Seal the edges of the petri dishes with a Parafilm™ strip or Petri Seal™ with the sterile surface next to the dish. Place in an environment with a temperature of 28 to 30°C under a 16-hour light/8-hour dark photoperiod. The cool, white fluorescent bulbs should be 20 to 25 cm away from the petri dishes.
2. Observe the changes in the embryos with and without endosperm for at least one week. Record your results and sketch the embryos. Indicate where most of the growth occurs and the type of growth.
3. Root and shoot growth may be measured aseptically by tracing their shapes with a thread on the surface of the closed petri dish and measuring the thread length.
4. Continue your observations by recording any changes in the developing embryos that are growing on the culture and water agar media.
5. At the end of the observation period, record the numbers of partial embryos showing shoot and/or root growth and compare them with the complete embryos.
6. Observe the changes in the embryos daily for one week. Measure and record root and shoot growth. Measure long, branched roots and estimate their lengths by using a 0.5-inch grid (Marsh, 1971). See Figure 4. Lay the roots in the lid of a petri dish. Add about 5.0 mL of water and tease the roots apart, so that they do not lie on top of one another. Invert the bottom of the petri dish and set it in the top so that it flattens the roots and keeps them from moving. Set the dish on the grid. Count every time a root crosses a vertical grid line, then count every time a root crosses a horizontal grid line. Add the 2 numbers and multiply by 10 to get the root length in millimeters.

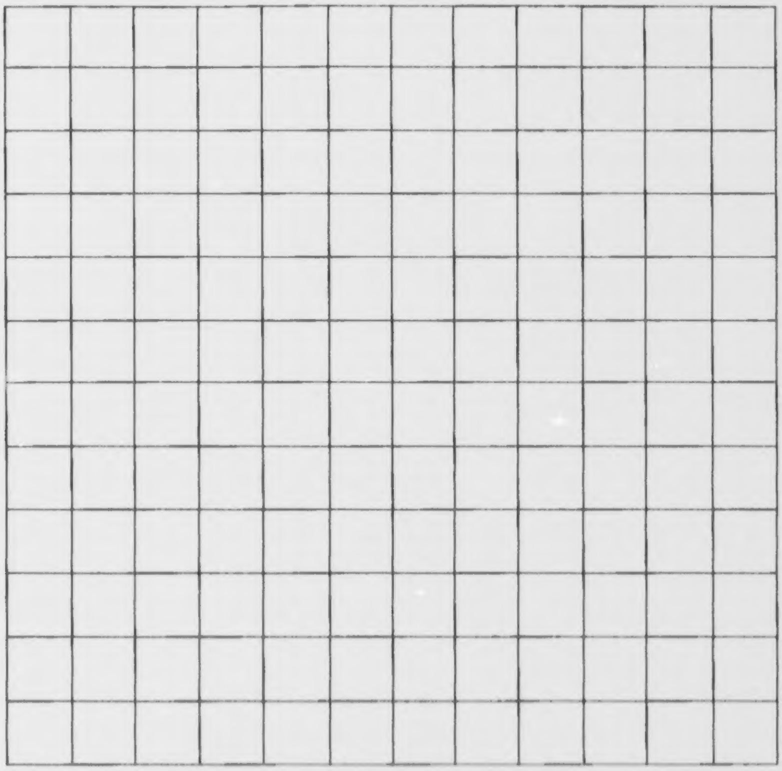

Figure 4. Grid.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 1. Growth of excised complete corn embryos on complete, artificial medium and embryos with endosperm on water agar after 5 days.

Replicate	Embryos with endosperm		Embryos with artificial medium	
	Length of shoot (mm)	Length of root (mm)	Length of shoot(mm)	Length of root (mm)
1	28	64	71	210
2	24	25	59	92
3	8	9	54	113
4	10	8	43	290
5	17	33	57	165

Appropriate display for these data is a frequency histogram of the total growth for each treatment. It is adequate, however, to describe these data with the mean for each group. The appropriate analytical technique is the statistical t-test. Here, however, the results are so dramatically different, that no analysis is necessary.

TEACHER'S NOTES

Interpretation

There is no overlap in these data. Embryos on artificial medium consistently grew more than embryos with endosperm provided. The overall mean growth for embryos to include shoot and root growth on artificial medium at 231 mm was 5 times that of embryos with endosperm at 45 mm. It appears that fresh endosperm may inhibit the growth of corn embryos.

Answer to Test Question

A

TEST QUESTION

One group of students recorded the following average growth measurements in the shoot and root of their excised corn embryos with and without endosperm.

Table 2. Growth of excised, complete corn embryos on M&S Basal Medium over 5 days.

Day	With endosperm		With artificial medium	
	Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)
1	1	2	1	3
2	2	3	2	6
3	4	4	4	12
4	6	8	8	27
5	8	18	18	53

A valid conclusion from their data would be:

A. The total root growth is greater than the total shoot growth regardless of the nutrient source.

B. The rate of growth was the same for both root and shoot with both nutrient sources.

C. The total shoot growth is greater than the total root growth regardless of the nutrient source.

D. The greatest amount of shoot and root growth occurred on Days 1 and 2.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think about questions that occurred to them as they conducted the Core Experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students might consider include the following:

1. What happens if only a partial embryo is grown on nutrient medium?
2. Does the orientation of the embryo or how it is cut determine what tissues develop?
3. Are there home "recipes" used for plant tissue culture?
4. How do variables, such as light and temperature affect plant growth?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to page 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

Investigations that require sterile technique in growing and transferring plant or animal material are not suitable for students who are visually impaired. This investigation takes considerable time and would be challenging to impossible to keep a student who is just "sitting by" interested in the work. The blind student and his project may be better located in a greenhouse, preparation room, or some place away from the area where students are using sterile techniques.

Deaf or Hard-of-Hearing

Modifications of this experiment for students who are hearing impaired or deaf are not necessary. As for most investigations, the main criterion is the ability of the student to communicate effectively with the instructor and with the laboratory partners.

Gifted

- Additional research will be required for students to predict the correlation between the type of hormone present in the growth medium and the differential growth of shoot or root.
- Embryos of other tissues, such as cucumber or melon, will provide a greater challenge for excision with this group of students.

Mobility Impaired

This is an investigation not suitable for students who are manually impaired.

- Invite other students who are mobility impaired to hold a conference with the instructor to determine the suitability of this investigation.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. What part of the complete seed performs the same function as the growth medium in the culture vessel?
   A. seed coat
   B. endosperm
   C. embryo
   D. coryledons
2. Not all embryos that were transplanted grew equally well. What are some factors that might explain this observation?
3. You are planning to grow embryos using agar cultures in the greenhouse. What are some factors that you must deal with to be successful?

REFERENCES AND SUGGESTED READINGS

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Dodds, J. H. & Roberts, L. W. (1985). *Experiments in Plant Tissue Culture.* Cambridge, MA: Cambridge University Press, Chs. 1-4, 11.

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Marsh, B. (1971). Measurement of length in a random arrangement of lines. *Journal of Applied Ecology, 8,* 265-267.

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TEACHER'S NOTES

Answers to Additional Test Questions

1. C
2. Cell damage during handling of the embryos could have resulted in poorer growth of some embryos. In addition, it is possible that both detected and undetected contamination could have inhibited or enhanced growth of some transplanted embryos.
3. To culture tissues in a greenhouse, the light period, shade, or heat will need to be extended to maintain a constant temperature, and some clean laboratory conditions will be needed to prepare the explants for culture.

BEST COPY AVAILABLE

Skoog, F. & Tsui, C. (1948). Chemical control of growth and bud formation in tobacco stem segments and callus culture in vitro. *American Journal of Botany, 35*, 782-787.

Stribling, J. M. (1983). Pioneers in plant tissue culture. *Carolina Tips, 46*(9), 33-35.

Vasil, V. & Vasil, I. K. (1982). Characterization of an embryogenic cell suspension culture derived from cultured inflorescences of *Pennisetum americanum* (Pearl millet, Gramineae). *American Journal of Botany, 69*, 1441-1449.

Vasil, V. & Vasil, I. K. (1982b). The ontogeny of somatic embryos of Pennisetum americanum, L.) K. Schum I. In cultured immature embryos. *Botany Gazette, 143*, 454-465.

POSSIBLE SOURCES OF MENTORS

- Local college or university botany professor
- Local research lab or industry specializing in plant tissue culture
- Cooperating research scientist: James A. Saunders, Ph.D., United States Department of Agriculture; Biotechnology Unit; Building 50, Room 100; Beltsville, MD 20705. Phone 301.504.7477; Fax 301.504.6478; E-mail: saund10449@aol.com.

VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation of that experiment. The following directions are intended only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

VARIATION 1

VARIATION 1

The Effect of Orientation of Embryos on Development of Roots and Shoots on Nutritionally Complete Growth Medium

SYNOPSIS

Students will compare the types and numbers of roots and/or shoots developed in fresh corn embryos placed vertically and horizontally on nutritionally complete growth medium.

ADDITIONAL MATERIALS

- 10 fresh corn embryos

HYPOTHESIS GENERATION

Question

Does the orientation of the embryo affect the amount of time required for development?

Answers to Questions and Analysis on Student Page

1. Corn embryos can grow without the rest of the seed. Excised embryos grew better than embryos kept with the rest of the seed.
2. Corn does not germinate immediately after harvest in the fall. It may be protected by something in the endosperm from germinating at a time when seedlings are unlikely to survive. This inhibition would be removed through maturation of the grain or by changing weather conditions in the spring.
3. Tissue culture provides constant moisture and nutrients in needed quantities and the necessary growth promoting temperature and photoperiod.
4. If entire plants can be grown from pieces of leaf, one would not have to wait for fruits to mature before starting another crop and all fruits that mature can be eaten, rather than saving some or replanting.
5. Conditions useful to establish and maintain aseptic cultures include still air, disinfecting of all surfaces, sealing culture containers, and good personal hygiene with hair tied back, no bracelets, and no long sleeves.

Sample Hypothesis

If corn embryos are oriented vertically with the radical end of the embryo in the medium rather than horizontally, then the roots and shoots will develop more quickly.

Rationale

Vertical orientation places the corn embryo in the position that it is expected to grow. No time is necessary to reorient.

Sample Experimental Procedure

Transfer to culture vessels

1. Obtain 2 sterile petri dishes. Label one "horizontal" and the other "vertical."
2. Follow the procedure of the Core Experiment for sterilizing the surface of the corn embryos.
3. Aseptically, orient 5 corn embryos horizontally on the petri dish of M&S Basal Medium labeled "horizontal." See Figure 5.

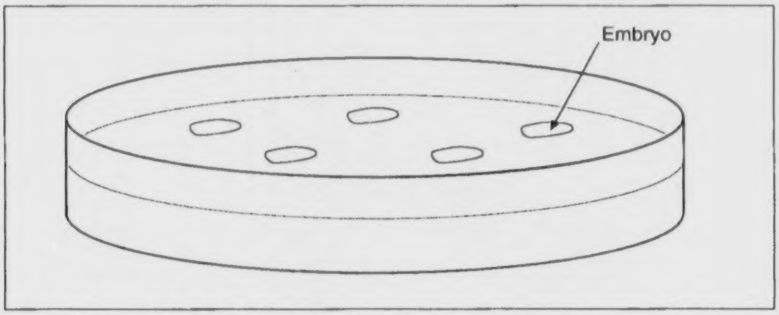


Figure 5. Corn embryos oriented horizontally.

4. Prepare the petri dishes and incubate the embryos with the light regime of the Core Experiment.
5. Aseptically, orient 5 corn embryos on the petri dish of M&S Basal Medium labeled "vertical." As you transfer these embryos, slit the agar with the scalpel and gently place each embryo vertically in a slit.

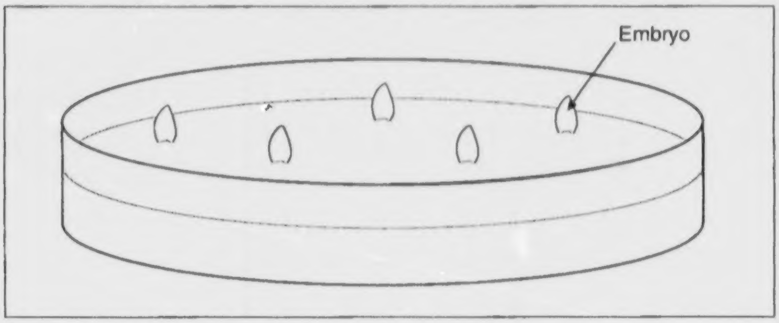


Figure 6. Corn embryos oriented vertically.

6. Prepare the petri dishes and incubate the embryos with the light regime of the Core Experiment.
7. Observe the changes in the "horizontal" and "vertical" embryos at room temperature for at least 1 week. Record your results and sketch the embryos, indicating where most of the growth takes place, and the type of growth that occurs.
8. Continue your observations by recording any changes in the developing embryos that are growing on the culture medium.
9. At the end of the observation period, record the numbers of "vertical" embryos showing normal shoot and/or root growth, as compared to the "horizontal" embryos.
10. Display and analyze these data.

TEACHER'S NOTES

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

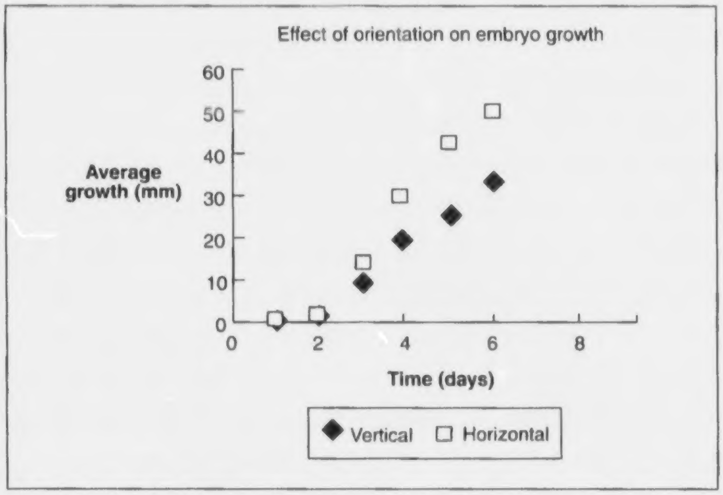
Table 3. Effect of embryo orientation on growth of excised embryos on nutritionally complete medium. Values reported are total lengths after 7 days of growth.

Replicate	Vertical		Horizontal	
	Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)
1	340	70	650	91
2	740	106	1210	105
3	310	108	520	79
4	230	60	560	107
5	350	81	520	97
6	470	94	630	81
7	210	93	860	102
8	250	62	520	97
9	930	113	1240	95
10	290	100	590	93

Total plant growth for each replicate and analyze the totals with a statistical t-test. An appropriate display of these data would be a frequency histogram.

TEST QUESTION

1. A group of students tested the effect of orientation on growth of excised corn embryos. They collected growth measurements daily. Their results are presented in Graph A. Write a conclusion for their experiment.



Graph A. Average growth of 5 corn embryos with different initial orientations.

2. Which grows faster in tissue culture: corn embryos laid on the surface of the medium, or corn embryos inserted vertically into the medium? Give two possible reasons for your answer.

Interpretation

Variations in the average total growth as great as found between horizontally and vertically oriented embryos are unlikely to be the result of chance alone. Reject the hypothesis and by inspection conclude that horizontally oriented embryos grew more than vertically oriented embryos.

Answer to Test Question

1. Embryos oriented horizontally consistently grew better than embryos oriented vertically.
2. Horizontally. Possible reasons include: gravity influences embryo growth and embryos are damaged by vertical implantation.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have students compare roots and shoots on young plants grown in plain and hydroponic solutions. This could be done by sighted students as well.

VARIATION 2

The Effect of Temperature on the Growth of Corn Seeds on a Nutritionally Complete Medium

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will compare the growth of corn embryos in vitro at different temperatures.

ADDITIONAL MATERIALS NEEDED

You will need the following:

- 1 refrigerator at 4°C
- 15 corn kernels
- 3 lights with timers
- 3 controlled temperature locations
- 3 petri dishes of M&S Basal Medium

HYPOTHESIS GENERATION

Question

How does temperature affect the growth of the embryo?

Sample Hypothesis

If corn seed embryos are grown at 28 to 30°C on a nutritionally complete growth medium, they will develop more quickly than embryos grown at 4°C on a nutritionally complete growth medium.

Rationale

Most plants begin to grow as the temperature increases in the spring even if they received adequate moisture when the temperature was decreasing in the fall.

Sample Experimental Procedure

1. Prepare 15 fresh corn embryos as directed in the Core Experiment.
2. Aseptically, transfer 5 embryos to each of 3 petri dishes containing M&S Basal Medium.
3. Incubate the embryos with the light regime of the Core Experiment at three different temperatures between 4°C (refrigerator) and 30°C.
4. Store dishes and make observations as directed in the Core Experiment.

TEACHING TIP

The temperature related change in growth resembles a response curve expected as a result of temperature effects on enzyme activity. Students can demonstrate that the cold, incubated plants have been stunted because their enzymes are inactive by bringing the petri dishes into the warm location and continuing the incubation. They should find that the growth rate increases in the new conditions.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 4. Effect of room temperature on growth of isolated corn embryos. Embryos were grown for 7 days.

Replicate	Total production (mm)		
	4°C	19°C	28°C
1	0	9	55
2	0	17	49
3	0	11	55
4	0	15	48
5	0	11	28

Use the means to describe these data. Display the results as a bar graph with the temperature as the independent variable on the x-axis and the mean length for each treatment as the dependent variable on the y-axis. Use standard deviation for error bars.

TEST QUESTION

1. How does temperature affect the growth of the excised embryo?
2. Design an experiment that would test whether light or temperature variations have the greatest effect on the total growth of excised embryos.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have students study the effect of temperature on the growth of seedlings in a nutritionally complete hydroponic solution. This may be done by sighted students as well.

VARIATION 3

The Effect of Kinetin and Adenine on the Growth of Corn Embryos in Vitro

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will compare the growth of corn embryos on a nutritionally complete medium with and without kinetin plus adenine added.

ADDITIONAL MATERIALS NEEDED

For the teacher preparation you will need the following for a class of 24:

- 10 mL 1N NaOH
- 200 mL distilled H_2O
- 2 mL 1N HCl
- 1 pH meter
- 6 sterile petri dishes

(Continued on p. 255)

Interpretation

These data support the hypothesis. There was no growth at 4°C. As the temperature was increased to 28°C, growth increased.

Answer to Test Question

1. Temperature extremes inhibit plant growth. At temperatures above 35°C enzyme function in cells will be adversely affected. At temperatures below 10°C enzyme function is significantly slowed, but the damage is not irreversible if the plant is brought back into a room temperature environment (20 to 25°C).
2. Using a nutritionally complete medium and one type of seed (embryo), set up culture vessels in various temperature conditions with normal light and in various light conditions with normal temperature. Record the growth (shoot + root) of the embryos daily. Average the growth for embryos in each of the various settings and compare them by plotting the average growth versus time (days) for each of the various settings on the same graph.

(continued from p. 254)

- 1 balance
- 5 g M&S Basal Medium with sucrose and agar (Carolina #195701)
- 0.01 g Kinetin (Carolina #F6 198353)
- 0.08 g Adenine (Carolina #F6 198331)

You will need the following for each group of two to four students in a class of 24:

- 1 sterile petri dish of Adenine Amended M&S Basal Medium
- 1 sterile petri dish of M&S Basal Medium
- 10 fresh corn kernels

DIRECTIONS FOR SETTING UP THE LAB

Kinetin Stock Solution

1. Dissolve 0.01 g of kinetin in 10 mL of 1N NaOH.
2. Add 8 mL distilled H_2O.
3. Store at 0°C.

Adenine Stock Solution

1. Dissolve 0.08 g of adenine in 2 mL of 1N HCl.
2. Add 8 mL distilled H_2O.
3. Store at 0 to 5°C.

Adenine-Kinetin Amended Medium

1. Add 4.24 g of M&S Basal Medium with sucrose and agar to 98 mL of room temperature distilled H_2O while gently stirring the water.
2. Stir continuously and heat until the solution becomes clear. Do not boil!
3. Add 1 mL Kinetin Stock Solution.
4. Add 1 mL Adenine Stock Solution.
5. Adjust the pH to 5.7 with 1N NaOH or 1N HCl.
6. Autoclave for 15 minutes at 15 pounds per square inch (psi).
7. Dispense to 6 sterile petri dishes.

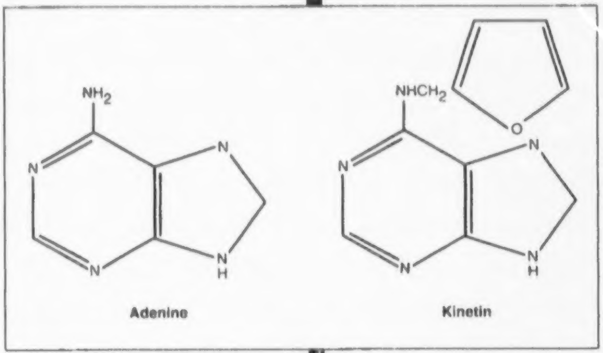


Figure 7. The structures of adenine and kinetin are similar.

HYPOTHESIS GENERATION

Question

What effect does the combination of the hormones kinetin and adenine have on embryo growth?

Sample Hypothesis

Fresh corn embryos grown in a nutritionally complete medium with kinetin plus adenine added will develop more shoots, as compared with embryos grown in a nutritionally complete medium without kinetin plus adenine added.

Rationale

A differential development favoring shoots has been shown for this kind of amendment with tobacco.

Sample Experimental Procedure

1. Prepare 10 corn embryos for tissue culture as in the Core Experiment.
2. Aseptically, transfer 5 embryos to M&S Basal Medium and 5 embryos to Adenine-Kinetin Amended Medium.
3. Incubate the embryos as in the Core Experiment.
4. After several days, measure and record the shoot and root growth.

TEACHING TIPS

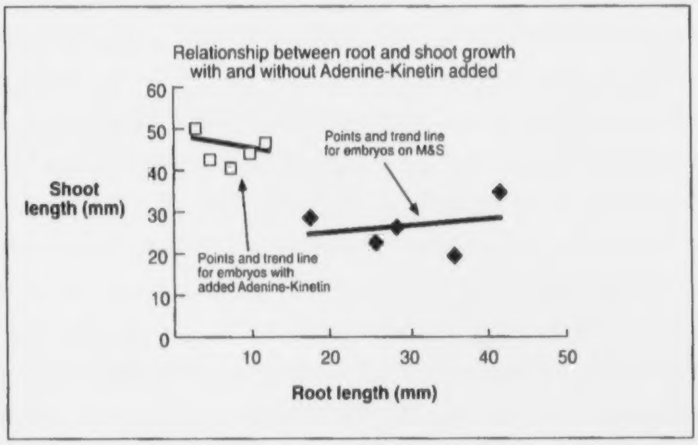
- Purchase medium containing the hormones for Variations 3 and 4. Adjusting the pH so that the agar sets up correctly is very time intensive and not always successful.
- Prepare stock enzyme solutions or purchase through a biological supply house: Kinetin (Carolina #F6 198353), Adenine (Carolina #F6 198331).
- The concept that a balance of common substances in plants controls differentiation is an economical mechanism. It is not necessary to postulate a different regulatory substance for each morphogenetic change in plants. Roots, shoots, sepals, petals, stamens, and pistils can be induced by regulating ratios of hormones rather than by developing different regulating substances.
- Some loss of kinetin activity occurs when it is autoclaved with other medium components, but that is only a concern in technical work.
- Adenine is structurally similar to kinetin and has kinetin activity. See Figure 7. The effect of adding adenine and kinetin should be similar to that of increasing kinetin concentrations.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Root and shoot production by excised corn embryos. Embryos were grown for 7 days.

Replicate	M&S Basal Medium		Adenine-Kinetin Amended Medium	
	Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)
1	26	28	41	3
2	25	26	39	5
3	30	18	42	8
4	35	42	49	2
5	20	35	43	11
Mean	27	30	43	6



Graph B. Effect of an adenine-kinetin addition to the medium is shown in the change in the relationship between roots and shoots.

TEST QUESTION

One group of students extended this exercise by testing to determine whether the Adenine-Kinetin Amended Medium could be used to clone corn from isolated roots of 4-day-old plants. Over 6 days, their roots on Adenine-Kinetin Amended Medium showed no increase in root length and no development of shoots. On the average, their roots on M&S Basal Medium grew 72% longer than their initial length, but no shoots developed. A second group wanted to know whether they could clone corn by using these media with isolated shoots from 4-day-old plants. They found that shoots on Adenine-Kinetin Amended Medium increased in length an average of 52%, but formed no roots. Shoots on M&S Basal Medium increased an average of 70% in length and 50% of these shoots developed single roots longer than 10 mm. Write a conclusion for these results.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have students study the growth of large plant embryos, such as avocado, with and without kinetin plus adenine. Sighted students may do this study as well.

Interpretation

The embryos were consistently smaller when they grew on Adenine-Kinetin Amended Medium. The ratio between roots and shoots for plants on M&S Basal Medium (1.13) is far greater than that for plants on Adenine Amended Medium (0.07). These data support the hypothesis.

Answer to Test Question

Both groups attempted to clone differentiated corn tissues. They found that the Adenine-Kinetin Amended Medium is not useful for cloning corn shoots or roots, at least at the concentrations used in a 6-day time frame. Although they found shoot growth and root development with their shoots of the M&S Basal Medium, they did not succeed in producing more plants. If this and the Core Experiment are the only information they have to draw on, they should conclude that cloning corn plants from 4-day-old plant tissue is not possible with these media.

- Provide molecular model kits to show the difference in chemical structure of adenine and kinetin.

VARIATION 4

The Effect of Kinetin-Indole-3-Acetic Acid (IAA) on the Growth of Corn Embryos in Vitro

***Note to Teachers:** In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.*

SYNOPSIS

Students will compare the growth of corn embryos on a nutritionally complete medium with and without the addition of kinetin and IAA.

ADDITIONAL MATERIALS NEEDED

For the teacher preparation you will need the following:

- 5 mL 1N NaOH
- 110 mL distilled H_2O
- 5 mL 1N HCl
- 1 pH meter
- 6 sterile petri dishes
- 1 balance
- 0.03 g Indole-3-Acetic Acid (IAA) (Carolina #F6 198250)
- 1 mL Kinetin Solution
- 1 autoclave
- 4.24 g M&S Basal Medium with sucrose and agar

You will need the following for each group of four students in a class of 24:

- 1 sterile petri dish of Kinetin-IAA Amended Medium
- 1 sterile petri dish of M&S Basal Medium with sucrose and agar
- 10 fresh corn kernels

SAFETY PROCEDURE

Use the liquid form of IAA where possible. If using powdered form of IAA, exercise caution when preparing solutions.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Kinetin Stock Solution

See Variation 3.

IAA Stock Solution (if not purchased)

1. Dissolve 0.03 g of IAA in 2 mL of 1N NaOH.
2. Add 8 mL distilled H_2O.
3. Store at -0°C.

Kinetin-IAA Amended Medium

1. Add 4.24 g of M&S Basal Medium with sucrose and agar to 98 mL of room temperature distilled H_2O, while gently stirring the water.

(continued on p. 258)

TEACHER'S NOTES

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TEACHING TIPS

- Nearly 50 years ago, Skoog and Tsui (1948, in Leopold, 1964) demonstrated that a balance between adenine and auxin determined which organs were formed from tobacco callus. Buds were produced when adenine was abundant and roots were produced when the auxin IAA was abundant. This medium should enhance the growth of shoots.
- Kinetin induces cell division in tobacco pith when IAA is also present. The optimum production of new cells occurs at kinetin concentrations less than 10 ppm (Skoog & Miller, 1957 in Leopold, 1964).

(continued from p. 257)

2. Stir continuously and heat until the solution becomes clear. Do not boil.
3. Add 1 mL Kinetin Stock Solution.
4. Add 10 mL IAA Stock Solution.
5. Adjust the pH to 5.7 with 1N NaOH or 1N HCl.
6. Autoclave for 15 minutes at 15 pounds per square inch (psi).
7. Dispense to 6 petri dishes labeled Kinetin-IAA Amended Medium.

HYPOTHESIS GENERATION

Question

How does the addition of IAA to kinetin affect embryo growth?

Sample Hypothesis

Fresh corn embryos grown in a nutritionally complete medium with kinetin plus IAA added will develop more roots, as compared with embryos grown in a nutritionally complete medium without kinetin and IAA.

Rationale

Students should provide their own rationale.

Sample Experimental Procedure

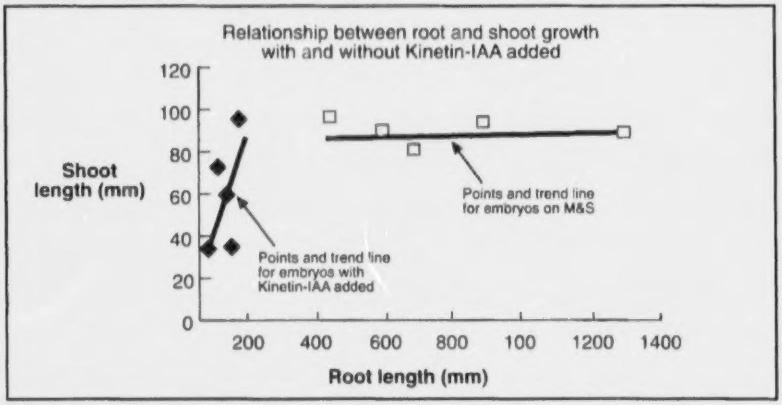
1. Prepare 10 fresh corn embryos as directed in the Core Experiment.
2. Aseptically, transfer 5 of the embryos to the dish containing the Basal Medium, as directed in the Core Experiment.
3. Aseptically, transfer 5 embryos to the dish containing the Kinetin-IAA Amended Medium.
4. Store dishes, observe and record data as directed to in the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Effect of Kinetin-IAA Amended Medium on shoot and root lengths.

Replicate	M&S Basal Medium		Kinetin-IAA Amended Medium	
	Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)
1	81	630	96	140
2	102	860	73	68
3	97	520	33	19
4	95	1240	61	82
5	93	590	37	115
Mean	94	768	60	85



Graph C. Effect of a Kinetin-IAA Amended Medium is shown in the change in the relationship between roots and shoots.

Interpretation

The embryos were consistently smaller when grown on Kinetin-IAA Amended Medium. The ratio between roots and shoots for plants on M&S Basal Medium (8.19) is far greater than that for plants on Kinetin-IAA Amended Medium (1.48). These data do not support the hypothesis.

TEST QUESTION

One group of students extended this exercise by testing whether Kinetin-IAA Amended Medium could be used to clone corn from the isolated roots of 4-day-old plants. Over 6 days, their roots on Kinetin-IAA Amended Medium showed no increase in root length and no development of shoots. On the average, their roots on M&S Basal Medium grew 72% longer than their initial length, but no shoots developed. A second group wanted to know whether they could clone corn from isolated shoots of 4-day-old corn plants by using these media. They found that on the average shoots on Kinetin-IAA Amended Medium increased in length 83%, but formed no roots. Shoots on M&S Basal Medium increased an average of 70% in length and 50% of these shoots developed single roots longer than 10 mm. Write a conclusion for these results.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variations 1, 2, and 3.

VARIATION 5

The Effect of Photoperiod on the Growth of Corn Embryos on a Nutritionally Complete Growth Medium

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Students will compare the growth of corn embryos on nutritionally complete medium when exposed to different photoperiods.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24:

2 identical temperature and humidity levels:
  - 1 24-hour per day light source
  - 1 12-hour per day light source

1 24-hour timer

You will need the following for each group of four students in a class of 24:

2 petri dishes of M&S Basal Medium

10 fresh corn kernels

HYPOTHESIS GENERATION

Question

What is the effect of a continuous light period on embryo growth?

Sample Hypothesis

If corn embryos are exposed to a continuous light period, then their total growth will be greater.

Rationale

Continuous light provides continuous opportunity for the plant to manufacture energy-rich compounds needed for growth.

Sample Experimental Procedure

1. Prepare 10 fresh corn embryos as directed in the Core Experiment.
2. Aseptically, transfer 5 embryos oriented horizontally to each of 2 petri dishes

(continued on p. 260)

Answer to Test Question

Both groups attempted to clone differentiated corn tissues. They found that the Kinetin-IAA Amended Medium is not useful for cloning corn shoots or roots, at least at the concentrations used in a 6-day time period. Although they found shoot growth and root development with their shoots of the M&S Basal Medium, they did not succeed in producing more plants. If this and the Core Experiment are the only information they have to draw on, they should conclude that cloning corn plants from sections of 4-day-old embryos is not possible with these media.

TEACHING TIPS

- The light source will also provide a heat source.
- An increase in root hair production, sometimes seen in this variation, is an indication of increased transpirational demand. If you can use the same light source for both photoperiods and provide a cover for the shorter exposures, you can avoid differences in temperature.
- The production of some plant growth regulators requires a dark period.
- Students may have collected data on growth with the Core Experiment photoperiod. They could use these data here as well.
- Plant growth chambers may be used to simulate the 12- and 24-hour photoperiods.

(continued from p. 259)

containing nutritionally complete medium. Seal dishes with Parafilm™, as directed in the Core Experiment.

3. Store 1 dish in an area that will receive 24 hours of continuous artificial light.
4. Store the other 3 dishes in an area that will receive 12 hours of continuous artificial light followed by 12 hours of darkness.
5. Make sure all dishes are kept at the same temperature and receive the same type of artificial light.
6. Make observations as directed in the Core Experiment.
7. Analyze these data.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 7. Total root and shoot length produced by corn embryos grown on M&S Basal Medium under different photoperiods for 7 days.

Replicate	Total root and shoot production (mm)	
	24-hour light	12-hour light
1	72	53
2	80	54
3	74	60
4	55	29
5	71	55
Mean	70	50

Appropriate analysis for these data is a statistical t-test. Appropriate display for these data is a frequency histogram, but because the sample size is small, an alternate display is acceptable.

TEST QUESTIONS

1. Compare the amount of growth in a corn embryo to the number of hours of light it receives each day.
2. The growth of each plant under the Core Experiment photoperiod was greater than that for any plant under these conditions. Provide a reasonable explanation for this observation.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variations 1, 2, and 3.

VARIATION 6

Growth of Dicots as Compared to Monocots on a Nutritionally Complete Growth Medium

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will compare growth of an isolated dicot embryo without its seed-food source with that of an isolated monocot embryo without its seed-food source.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 10 fresh or soaked pea, squash, or bean seeds

(continued on p. 261)

Interpretation

We conclude that results like ours are likely to occur by chance alone. There is no significant difference in the growth response of corn embryos under these two conditions. These data do not support the hypothesis.

Answers to Test Questions

1. The more hours of light an embryo gets each day, the longer it grows.
2. Answers will vary. Possible explanations include: The lab temperature this week was probably less than it was the week we performed the Core Experiment or the variety of corn used this week was different than the variety used for the Core Experiment. Some critical growth-regulating hormone is only produced during long days, and requires a dark period for production.

(continued from p. 260)

- 1 sterile foil-covered 250-mL beaker
- 100 mL 10.0% bleach-soap solution
- 2 sterile forceps
- 2 sterile scalpels with blades
- 5 fresh corn kernels
- 2 sterile petri dishes of M&S Basal Medium

HYPOTHESIS GENERATION

Question

How does the removal of the seed-food source of monocot and dicot affect their growth?

Sample Hypothesis

If the seed-food source of a dicot is removed, then the dicot will grow less vigorously than a monocot with its seed-food source removed.

Rationale

The cotyledons (dicot seed-food source) provide a continuous food supply for the plant because they photosynthesize.

Sample Experimental Procedure

1. Prepare corn embryos for tissue culture as in the Core Experiment.
2. Aseptically, transfer 5 embryos to petri dishes of M&S Basal Medium labeled "corn."
3. Prepare the petri dish and incubate the embryos with the light regime of the Core Experiment.
4. Use the soiled scalpel and forceps to practice excising dicot embryos from 5 pea seeds.
   a. Cut and remove the seed coat.
   b. Separate the cotyledons by slipping the scalpel blade between them and twisting it.
   c. Slide the scalpel blade along the cotyledon to where the remainder of the embryo is attached and cut the embryo from the cotyledon.
5. Sterilize the surface of the dicot seeds in bleach solution for 10 to 50 minutes if they are fresh and cleaned. If you are using dried seeds, soak them in water for 1 hour to overnight before surface sterilizing them.
6. Use the procedures you perfected in practice and aseptic techniques to extract the dicot embryos. Transfer 5 embryos to a second petri dish of M&S Basal Medium.
7. Seal the petri dishes with Parafilm™ or PetriSeal™ and incubate the pea embryos with the corn embryos.
8. After several days, measure and record the growth of shoots and roots on the embryos.
9. Display and analyze these data.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 8. Growth of excised dicot and monocot embryos with only tissue culture nutrients grown for 13 days.

Replicate	Corn			Peas		
	Root length (mm)	Shoot length (mm)	Total plant (mm)	Root length (mm)	Shoot length (mm)	Total plant length (mm)
1	720	106	730	15	3	18
2	310	108	418	15	2	17
3	260	60	320	12	2	14
4	250	81	331	11	6	17
5	470	94	564	14	3	17
Mean			473			17

(continued on p. 262)

TEACHING TIPS

- You can use dried seeds or fresh seeds, but fresh seeds are easier to work with because they are already soft. Dried seeds should be soaked for 2 days with a change of sterile water after the first day before they are as soft as fresh seeds.
- Produce from the supermarket is a good source of these materials.
- Squash seeds should be exposed to the bleach for 20 to 50 minutes rather than for only 10. To remove the seed coat from squash seeds, cut through it inside the ridge around the edge, except at the pointed end. Use the scalpel blade to lift the seed coat and push out the embryo. A squash embryo can be separated from the cotyledons by simply cutting off 1 to 2 mm at the pointed end.
- It is easier to dissect and locate the embryo in bean seeds than in either corn or cucumber seeds. If you choose to use bean seeds, have students surface sterilize them as they did the corn seeds in the Core Experiment. Students will need to remove the seed coat from the dicot seed and to separate the embryo from the cotyledons. Be careful to excise both the radicle (root) and the shoot of the bean seed. You will find it "sandwiched" between the cotyledons. Forceps can be used for this dissection.

Interpretation

Pea embryos grew very poorly when tissue culture medium was substituted for cotyledons. Perhaps another medium formulation would produce good growth, but this medium does not appear to replace the contribution of the cotyledons. Additional testing to determine how well the peas would grow in these conditions when the embryo was intact should be used to compare the growth of embryos on tissue-culture medium. Corn grew much better than peas, so our hypothesis is supported by these data.

(continued from p. 261)

The appropriate display for these data is a frequency histogram. The display here is not a frequency histogram because the data sample is small. Appropriate analysis here would be the statistical t-test, but since there is no overlap in these data, a test is not necessary.

TEST QUESTION

Another group of students grew winter squash embryos and compared the growth of complete embryos on 7% water agar with embryos without cotyledons on M&S Basal Medium. Their data are presented in Table 9. Is M&S Basal Medium a good substitute for the cotyledons? How do you know?

Table 9. Winter squash growth with nutrients supplied by the cotyledons or by M&S Basal Medium after 6 days. Shoot growth is measured to the tip of the base of the cotyledons or to the base of the unexpanded first true leaves.

Replicate	Complete embryos		Incomplete embryos	
	Root growth (mm)	Shoot growth (mm)	Root growth (mm)	Shoot growth (mm)
1	137	11	60	5
2	97	12	22	5
3	267	16	30	5
4	126	8	28	5
5	70	8	20	5

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variations 1, 2, and 3.

VARIATION 7

The Effect on Embryo Development of Cloning by Longitudinally Dividing an Excised Fresh Corn Embryo

Note to Teachers: *In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.*

SYNOPSIS

Students will compare the growth produced by complete fresh corn embryos on a nutritionally complete growth medium with the growth produced by longitudinally divided embryos.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two to three students:

- 4 sterile petri dishes of M&S Basal Medium
- 10 fresh corn kernels

HYPOTHESIS GENERATION

Question

What effect does dividing the embryo longitudinally have upon its growth?

Answer to Test Question

The M&S Basal Medium is not an adequate substitute for cotyledons for winter squash. The embryos that retained their cotyledons grew an average of 4 times larger than those that had their cotyledons removed.

TEACHING TIPS

- Students may want to compare shoot to root ratios. There is no intrinsic reason to expect a difference. Collecting root and shoot lengths rather than just total growth will help them see this.
- Here the expectation should be focused on quantitative difference (how much it grew) rather than on qualitative (how it grew).
- Although the hypothesis relates to the rate of growth, it is not necessary to make daily measurements. If all embryos are grown for the same length of time, total production is an adequate measure of growth rate.

Sample Hypothesis

If an embryo is divided longitudinally, then it will develop more slowly than a complete embryo.

Rationale

The part of the embryo that would have developed a shoot is ready to do so, and the part that will develop a root is ready to do so. However, the longitudinal half-embryo has only half as many cells of each kind as the complete embryo.

Sample Experimental Procedure

1. Label 2 sterile petri dishes with M&S Basal Medium "complete" and 2 sterile petri dishes "longitudinal."
2. Prepare 10 corn embryos for tissue culture as in the Core Experiment.
3. Aseptically, transfer 5 complete embryos to each of 2 petri dishes of M&S Basal Medium.
4. Seal the petri dishes and incubate the embryos with the light regime of the Core Experiment.
5. Aseptically, cut the remaining corn embryos in half longitudinally.
6. Aseptically, transfer 5 of the longitudinal half-embryos to each of 2 sterile petri dishes of M&S Basal Medium.
7. Seal the petri dishes and incubate the embryos with the light regime of the Core Experiment.
8. After several days, measure and record the shoot and root lengths produced by each embryo and half-embryo.
9. Display and analyze these data.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 10. Growth measurements of excised corn embryos after 13 days on tissue culture medium.

Replicate	Longitudinal half-embryos			Complete embryos		
	Root length (mm)	Shoot length (mm)	Total growth (mm)	Root length (mm)	Shoot length (mm)	Total growth (mm)
1	420	89	519	660	91	751
2	440	100	540	1210	105	1315
3	340	80	420	520	79	599
4	160	66	226	560	107	667
5	230	83	313	520	97	617
6	430	111	541	630	81	711
7	300	101	401	860	102	962
8	510	100	610	520	97	617
9	0	9	9	1240	96	1336
10	0	6	6	590	93	680

TEACHER'S NOTES

Interpretation

Statistical analysis reveals that the growth of half and complete embryos differs. We accept the hypothesis. Inspection of these data reveals that complete embryos grew faster than half embryos.

Answer to Test Question

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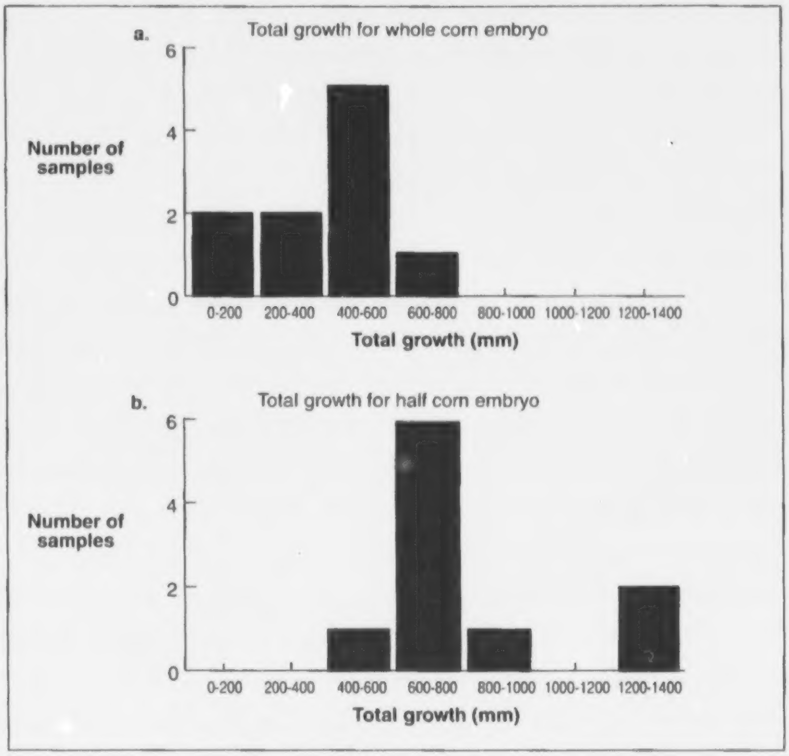

Graph D. Total growth for whole (a) and partial (b) corn embryos grown for 13 days.

TEST QUESTION

One group of students recorded the following data for an exercise in which they wanted to compare growth of embryos.

Table 11. Growth of partial and complete corn embryos over 3 days.

Time (days)	Partial embryos		Complete embryos	
	Average shoot length (mm)	Average root length (mm)	Average shoot length (mm)	Average root length (mm)
1	2	1	2	3
2	3	2	3	6
3	4	6	4	12

A valid conclusion from their data would be:

A. Total root growth is greater than total shoot growth for both partial and complete embryos.

B. The rate of growth was the same for both root and shoot for partial and complete embryos.

C. Total root growth is greater than total shoot growth for both partial and complete embryos.

D. The greatest amount of growth occurred on the second day.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variations 1, 2, and 3.

VARIATION 8

The Effect on Embryo Development of Cloning by Transversely Dividing an Excised Fresh Corn Embryo

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will compare the shoot to root ratio developed by complete fresh corn embryos on a nutritionally complete growth medium with the ratio developed by transversely divided embryos.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students:

- 4 sterile petri dishes of M&S Basal Medium
- 20 corn embryos

HYPOTHESIS GENERATION

Question

What is the effect on embryo growth if the embryo is divided transversely?

Sample Hypothesis

If an embryo is divided transversely, then it will develop roots or shoots differently than an undivided embryo.

Rationale

The part of the embryo that would have developed a shoot is ready to do so, but new cells need to be formed and oriented for producing a root. Therefore, the shoot half should develop shoot more quickly than root. The reverse might be expected for the root half.

Sample Experimental Procedure

1. Label 2 sterile petri dishes "complete" and 2 additional sterile dishes "transverse."
2. Prepare corn embryos for tissue culture as in the Core Experiment.
3. Aseptically, transfer 5 complete embryos to each of 2 petri dishes of M&S Basal Medium labeled "complete."
4. Seal the petri dishes and incubate the embryos with the light regime of the Core Experiment.
5. Remove 5 corn embryos and cut them in half transversely.
6. Aseptically, transfer 5 of the transverse half-embryos to each of 2 petri dishes of M&S Basal Medium labeled "transverse."
7. Seal the petri dishes and incubate the embryos with the light regime of the Core Experiment.
8. After several days, measure and record the shoot and root lengths produced by each embryo and half-embryo.
9. Display and analyze these data.

TEACHING TIPS

- Students may want to compare total growth. They certainly should expect to see a difference since they have reduced the number of starting cells by approximately half.
- Here the expectation should be focused on qualitative difference (how it grew) rather than on quantitative difference (how much it grew).

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DATA ANALYSIS AND INTERPRETATION

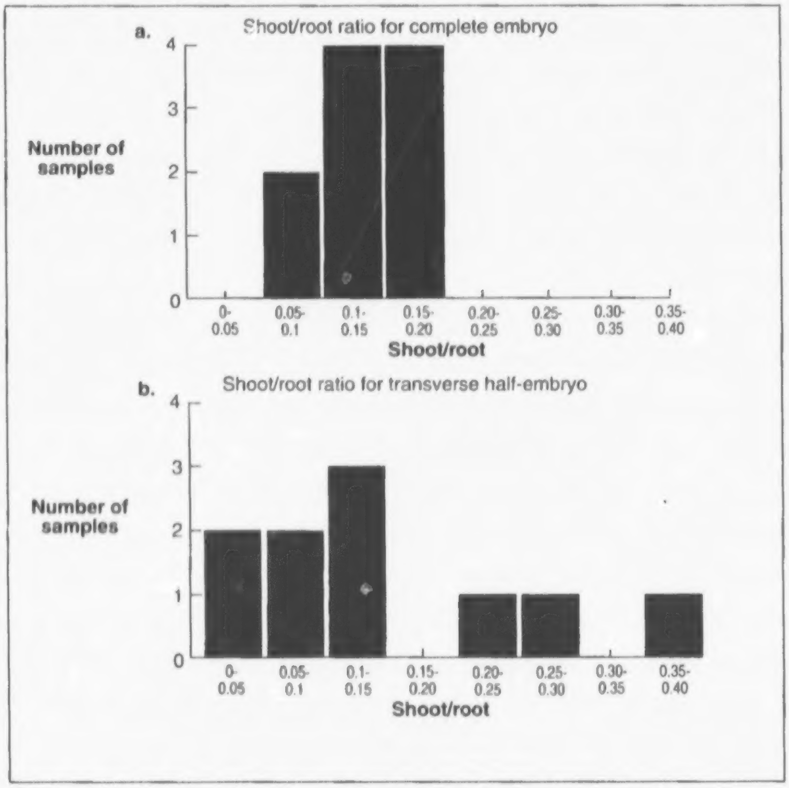
Sample Data

Table 12. Growth measurements of excised corn embryos after 13 days on a tissue culture medium.

Replicate	Transverse half-embryos			Complete embryos		
	Root length (mm)	Shoot length (mm)	Shoot/root	Root length (mm)	Shoot length (mm)	Shoot/root
1	610	89	0.15	660	91	0.14
2	730	76	0.10	1210	105	0.09
3	950	93	0.10	520	79	1.15
4	410	0	0	560	107	0.19
5	680	104	0.15	520	97	0.19
6	690	0	0	630	81	0.13
7	170	50	0.29	860	102	0.12
8	380	75	0.20	520	97	0.19
9	550	70	0.13	1240	96	0.08
10	210	75	0.36	590	93	0.16

Interpretation

Although our half embryos grew more slowly than our complete embryos, their growth was not different than that produced by complete embryos. Reject the hypothesis and accept the notion that values as different as those observed are likely to occur by chance alone.



Graph E. Distribution of growth among complete (a) and transverse half (b) embryos.

TEST QUESTION

Although the complete embryos and half embryos grew at different rates. They did not produce statistically different shoot to root ratios. What does this tell you about the genetic potential of embryonic corn cells?

Answer to Test Question

Each cell in a corn embryo must have the ability to develop into either shoot or root tissues, because each transverse half was able to make a complete embryo.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- This variation is not suitable to copy in larger size.

VARIATION 9

The Effect of Endosperm to Embryo Ratio on the Growth of Grass Embryos on a Nutritionally Complete Medium

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS

Students will determine if grass species with different amounts of endosperm provided relative to the embryo size will produce different growth responses in tissue culture.

APPROPRIATE BIOLOGY LEVEL

Advanced

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- 300 mL of 10% bleach-soap solution
- 3 sterile, foil-covered 250-mL beakers
- 3 petri dishes of M&S Basal Medium
- 30 seeds of each of 3 or more kinds of grass

HYPOTHESIS GENERATION

Question

What is the effect of endosperm to embryo ratio on embryo growth?

Sample Hypothesis

If a grass has a small endosperm to embryo ratio, then it will grow long more quickly than if it has a large endosperm to embryo ratio.

Rationale

An embryo provided with little endosperm needs to produce its own food promptly. The faster the shoot reaches the light and leaves expand, the sooner the plant can produce its own food.

Sample Experimental Procedure

TEACHING TIPS

- Students can collect their own grass seeds for this exercise or you can obtain seeds from a health food store or feed store.
- Accessory floral structures adhere to some grass seeds and should be removed before the seeds are surface sterilized. The extra tissues make it more difficult to sterilize the seeds and more difficult to remove the embryo. Soak the seeds for an hour in 30°C water before removing the floral parts.
- If students practice removing embryos under magnification with a dissecting microscope, they will be able to judge the right amount of seed to remove the embryos without magnification when they need to excise them aseptically.
- The basic structure of grass flowers is a floret. It consists of the flower enclosed in a lower bract (the lemma) that encloses an upper bract (the palea) and the flower. Several florets are combined in a spikelet and usually several spikelets in a more complex inflorescence.
- Figure 8 is provided to help you guide your students in removing the accessory tissues, not to create a vocabulary lesson in grass flower structure. The parts to be removed are what Garrison Keillor refers to as "oat hulls and wheat chaff."

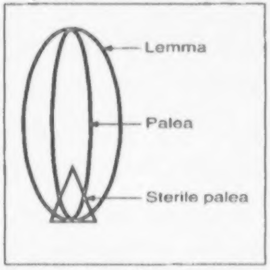


Figure 8. An unmilled grass seed will look like this diagram. The embryo is on the far side of the seed, opposite the surface showing in this diagram.

- All of the grasses used here have a groove opposite the side where the embryo is located.

TEACHER'S NOTES

Sample Experimental Procedure

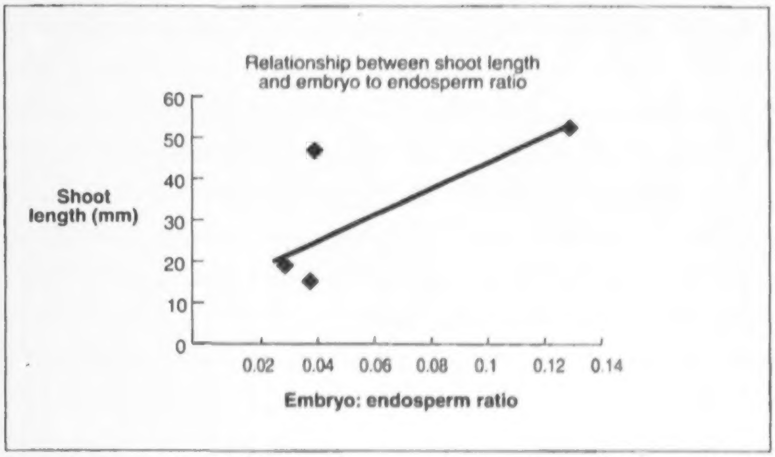
1. Soak seeds for about 1 hour in 30°C tap water to soften the accessory floral parts before removing them. Discard any seeds that have begun to germinate.
2. Sterilize the surface of seeds as in the Core Experiment.
3. Excise the embryos by cutting them away from the endosperm.
4. Transfer 5 embryos of each kind to a separate petri dish of M&S Basal Medium.
5. Incubate the excised embryos as in the Core Experiment.
6. After several days, measure and record the shoot and root lengths produced.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 13. Growth of various grasses in tissue culture. Plants were grown for 7 days. The average endosperm/embryo ratios were obtained by drying several excised embryos and their endosperms. The pericarp was included with the endosperm except for corn. The sample size was 20 seeds except in the case of wheatgrass, where 45 seeds were used.

Grass genus	Replicate	Shoot length (mm)	Root length (mm)	Endosperm/ embryo
Zea (corn)	1	71	210	0.128
	2	59	92	
	3	54	113	
	4	43	290	
	5	57	165	
Agropyron (wheatgrass)	1	27	32	0.033
	2	25	20	
	3	17	38	
	4	20	46	
	5	11	21	
Triticum (wheat)	1	23	25	0.038
	2	12	17	
	3	15	36	
	4	23	33	
	5	12	20	
Hordeum (barley)	1	53	27	0.039
	2	51	36	
	3	50	56	
	4	28	60	
	5	40	30	



Graph F. The relationship between shoot growth and endosperm/embryo ratio. All plants were grown for 7 days.

TEST QUESTION

Assuming that the relationship developed in your graphical presentation is correct, how long would you expect the shoot of a grass with an embryo to endosperm ratio of 0.085 to be in 7 days?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 8.

VARIATION 10

The Effect of Alternate Basic Tissue Culture Medium on the Growth of Complete Corn Embryos

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.

SYNOPSIS

Students will compare the growth of isolated corn embryos on a commercial medium with their growth on a simplified medium.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24:

- 6 mL 10-10-10 water soluble fertilizer
- 1.5 L distilled H_2O
- 720 mL table sugar
- 2 500-g inositol tablets
- 2 multivitamin tablets containing thiamine
- 60 g agar flakes
- citric acid
- bicarbonate of soda

Interpretation

It was predicted that the wheatgrass, wheat, and barley, with low embryo to endosperm ratios, would have more rapid shoot growth than corn when all of the embryos were supplied with the same nutrient source. These data do not support the hypothesis. Of the embryos tested, corn has the most rapid shoot growth. The correlation (R^2= 0.7413) between shoot length and the embryo to endosperm ratio is not strong. Additional data points would be useful to further support this hypothesis or to refute it.

Answer to Test Question

The shoot of this mystery grass grown from an isolated embryo should be 43 mm long in 6 days.

TEACHING TIPS

- This recipe for tissue culture is from the Internet Site: http://www.une.edu.au/-agronomy/AgSSrHortTCinfo.html. It calls for rain water, but distilled water is available in supermarkets and has been substituted here. Multivitamins come with a wide variety of thiamine amendments. Those used to obtain these data contained 10 mg and minerals not specified in the recipe. The original recipe also called for fertilizer with a 10:10:10 formulation not commonly available here. The volume of solutions was adjusted to arrive at the correct concentrations.
- In the early 1940's coconut milk was introduced to promote embryo growth in tissue culture. Amending this formulation with coconut milk could provide interesting results. Encourage your students to speculate on the cause of the growth increase, particularly if they have investigated effects of specific hormones. To make the amendment, add 1 cup of coconut milk and 1 teaspoon of malt.

TEACHER'S NOTES

You will need the following for each group of three students in a class of 24:

- 1 sterile petri dish of Market Tissue Culture Medium
- 1 sterile petri dish of M&S Basal Medium
- 10 fresh corn kernels

DIRECTIONS FOR SETTING UP THE LAB

Fertilizer Stock

Add 6 mL of 10:10:10 (N:P:K) water soluble fertilizer to 1 L distilled water.

Market Tissue Culture Medium

1. Combine
   - 480 mL distilled water
   - 240 mL Fertilizer Stock
   - 720 mL table sugar
   - 2 500-mg inositol tablet, crushed
   - 2 thiamine-containing multivitamin tablets, crushed
   - 4 tablespoons agar flakes
2. Boil gently, stirring constantly, until the agar dissolves. Use citric acid or bicarbonate of soda to adjust the pH to between 5 and 6. Autoclave and dispense this agar the same way you autoclaved and dispensed the M&S Basal Medium.

HYPOTHESIS GENERATION

Question

Do isolated embryos have specific nutrient requirements for growth?

Sample Hypothesis

If isolated corn embryos are grown on medium other than M&S Basal Medium, they will not grow as quickly.

Rationale

Commercial media prepared with purified ingredients is usually formulated for culturing specific species. General-purpose medium of common ingredients is unlikely to support vigorous corn growth because corn is not a species hobby horticulturists are likely to culture.

Sample Experimental Procedure

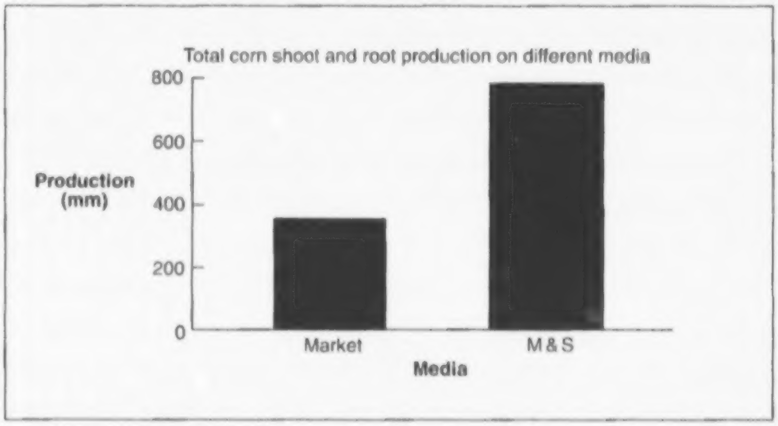
1. Surface sterilize seeds following directions of the Core Experiment.
2. Using the procedures of the Core Experiment, extract embryos and place 5 embryos horizontally on M&S Basal Medium and 5 embryos on Market Tissue Culture Medium.
3. Seal the petri dishes with Parafilm™ or PetriSeal™ and place the dishes under lights as done in the Core Experiment.
4. Calculate the total length of roots and shoots over a period of several days.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 14. Total shoot and root growth produced by whole corn embryos during 13 days incubation with a photoperiod of 16 hours light/8 hours dark.

Replicate	Medium type	
	Market	M&S
1	155	599
2	280	617
3	288	667
4	450	741
5	490	1315
Mean	333	788



Graph G. The production of corn embryos on different media after 13 days is represented by the total length of roots and shoots. Each bar represents the average growth for 5 plants.

Interpretation

The hypothesis is supported by these data. Plants grown on the commercially formulated medium (M&S) produced more total growth than did plants grown on the Market Tissue Culture Medium (Market). The average growth on M&S medium was more than twice that on the Market Medium.

TEST QUESTION

Another group of students decided that they were concerned about the ability of corn grown on Market Medium to produce a root/shoot ratio that was typical of plants nourished by endosperm. They obtained the following data. Determine the root/shoot ratios and compare these values.

Table 15. Root and shoot growth produced by corn embryos with different nutrient sources.

Replicate	Market Medium			Endosperm present		
	Root length(mm)	Shoot length	Root/shoot length(mm)	Root length(mm)	Shoot length	Root/shoot length(mm)
1	240	40		64	28	
2	130	25		25	24	
3	250	38		9	8	
4	400	50		8	10	
5	430	60		33	17	

Answer to Test Question

Table 16. A comparison of the root to shoot ratio produced by corn grown with different nutrient sources.

Replicate	Market Medium			Endosperm present		
	Root length(mm)	Shoot length	Root/shoot length(mm)	Root length(mm)	Shoot length	Root/shoot length(mm)
1	240	40	6.0	64	28	2.3
2	130	25	5.2	25	24	1.0
3	250	38	6.6	9	8	1.1
4	400	50	8.0	8	10	0.8
5	430	60	7.2	33	17	1.9

These results clearly show that root growth is retarded relative to shoot growth by fresh endosperm. The average root/shoot ratio in market medium (6.6) is 4.7 times greater than the average ratio (1.4) with endosperm present.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 8.

Plant Tissue Culture: Embryo Isolation and Tissue Culture Initiation

Directions for Students

INTRODUCTION

Imagine that you are chosen to be a member of the food production team aboard the first Spacelab and that your responsibility is to manage the growth of plants to support life in the self-contained environment. What technique(s) could you use to produce food quickly and without soil?

The commercial use of plant tissue culture is a relatively recent technique for growing plants quickly. Several types of culture techniques have been developed for the specific purposes of micropropagation, cloning of plants with desirable characteristics, and nutritional studies in a controlled environment. One technique that is easily demonstrated in the lab is excising or "cutting" an embryo from the seed and growing it on a sterile growth medium. See Figure 1. The advantages to this technique are:

- more rapid growth of the embryo
- more plants can be grown in a small space, and
- specific nutrient requirements and physical conditions affecting the plant can be easily controlled and modified.

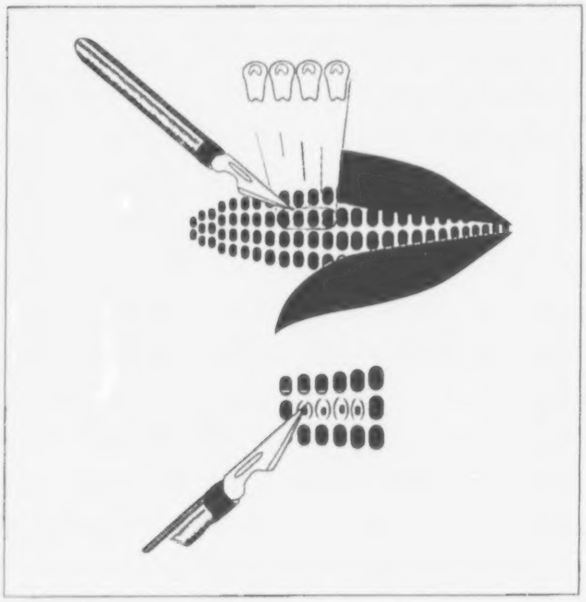


Figure 1. Excising the embryo from a corn kernel.

By removing the embryo from the seed and growing it in a culture vessel (in vitro), you are altering its natural environment. What conditions must be provided for the embryo to grow successfully in vitro?

OBJECTIVES

At the end of this lab you should be able to:

- Excise a plant embryo from a seed.
- Inoculate a sterile, nutritionally complete growth medium with the excised embryo.
- Observe the growth of a plant embryo in vitro.
- Describe the effects of specific hormones on plant embryo growth in vitro.

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SAFETY NOTES

Safety goggles and lab aprons/coats are to be worn at all times when working with chemicals, heat, and glassware.

Use care when working with sharp instruments. Cut away from your body when using scalpels.

Maintain sterile conditions at all times in the work area.

Wash hands with antibacterial soap as instructed throughout the lab procedure and before leaving the lab.

At the conclusion of the lab exercise, all living materials must be autoclaved before disposing to prevent exposure to accidentally introduced pathogens.

Keep open containers of alcohol away from flames.

MATERIALS NEEDED

You will need the following for each group of two to four students in a class of 24:

- 1 250-mL spray bottle of 70% ethanol or 10% household bleach
- 1 sterile petri dish with M&S Basal Medium with sucrose and agar
- 1 sterile petri dish with water agar
- 1 permanent marking pen
- 1 bottle of antibacterial soap
- 1 fresh supermarket ear of yellow corn (*Zea mays*) or dry field corn seeds
- 50 mL sterile water
- 25 mL 10% household bleach-soap solution
- 3 sterile, disposable petri dishes
- 1 sterile film canister or 1 250-mL beaker with aluminum foil cover
- 1 timer
- 1 shallow pan
- 1 sterile surgical scalpel, Bard-Parker No. 3 handle
- 1 sterile No. 10 blade
- 1 sterile No. 11 blade
- 2 sterile forceps, 11-cm fine point
- 1 500-mL beaker
- 2 sterile paper towels
- 2 2.5 x 15-mm Parafilm™or Petri Seal™ strips

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature or web search on the topic of plant tissue culture. Summarize your findings and cite your references. Your teacher may be able to suggest some journals.

HYPOTHESIS GENERATION

From the information you have on this topic develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis.
Answer the following questions:

1. What is the question you are investigating?
2. What makes this question an interesting or important topic for investigation?
3. What additional variables need to be controlled for? How will you accomplish this? Why is it important to control for these variables?

PLAN OF INVESTIGATION

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedure you will follow. Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure? How will you make these measurements?
3. How can you show your results in a graph?

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

1. Can just an embryo grow without the rest of the seed?
2. Usually, corn left on the ground in fall does not begin growing until the following spring. How can you overcome the delay in the ability of the corn to grow?
3. What factors does tissue culture provide for the embryo's survival?
4. Even differentiated tissues like leaves and roots can be induced to produce whole plants. How might this ability be beneficial in a space station?
5. What procedures are necessary or helpful to maintain aseptic cultures?

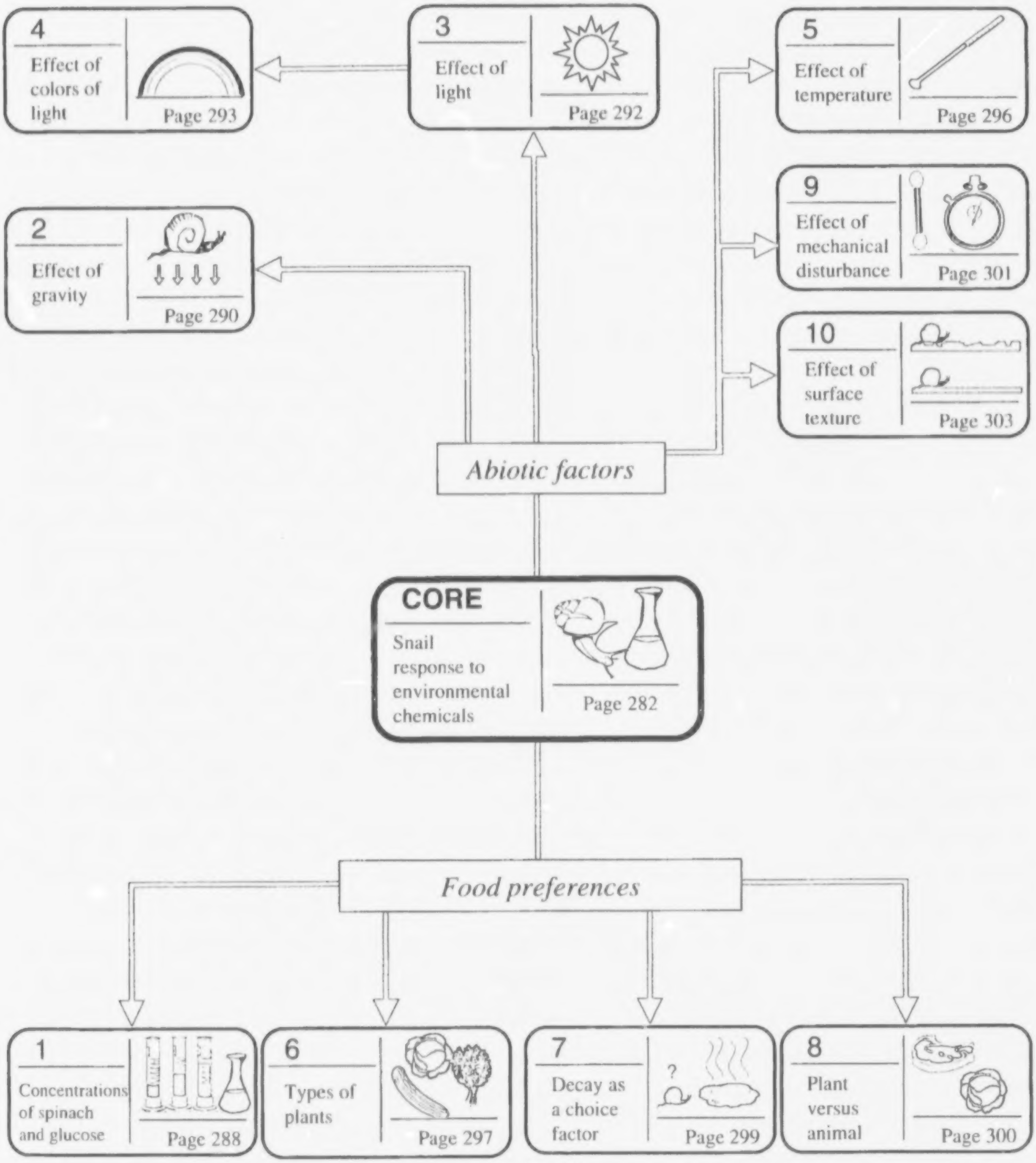
DESIGN OF VARIATIONS OF CORE EXPERIMENT

- Does the orientation of the embryo affect the amount of time required for development?
- How does temperature affect the growth of the embryo?
- What effect does the combination of the hormones kinetin and adenine have on embryo growth?
- How does the addition of IAA to kinetin affect embryo growth?
- What is the effect of a continuous light period on embryo growth?
- How does the removal of the seed-food source of monocot and dicot affect their growth?
- What effect does dividing the embryo longitudinally have upon its growth?
- What is the effect on embryo growth if the embryo is divided transversely?
- What is the effect of endosperm to embryo ratio on embryo growth?
- Do isolated embryos have specific nutrient requirements for growth?

NOTES

A Study of Snail Behavior

At-A-Glance Map of the Core Experiment and Its Variations


4
Effect of colors of light
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3
Effect of light
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Effect of temperature
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Decay as a choice factor
?
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Plant versus animal
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NOTES

A Study of Snail Behavior

SYNOPSIS FOR CORE EXPERIMENT

Students will determine if land snails, genus *Helix*, respond to different chemical stimuli in their environment by observing their behavior.

APPROPRIATE BIOLOGY LEVEL

Introductory or advanced

TEACHER PARTNERS

Diana Reinhard
and Peter Stallone
Springbrook High School
201 Valley Brook Drive
Silver Spring, MD 20904

SCIENTIST PARTNER

Dr. Wallace Pickworth
National Institutes of Health
National Institute on Drug Abuse
Addiction Research Ctr., Box 5180
Baltimore, MD 21224

Directions for Students

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 288. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Determine how snails respond to chemical stimuli, including chemicals found in their environment.
- Discuss why snails may respond differently to various chemical stimuli in their environment in terms of evolution.

MATERIALS NEEDED

For the teacher preparation, you will need the following for a class of 24:

- 1 10-gallon terrarium or 40 x 22 x 25-cm (16 x 9 x 10-inch) plastic storage box with lid
- 10 g calcium carbonate ($CaCO_3$) chips or 1 cuttlebone
- 500 mL pond or aged spring water
- 1 10-cm jar lid or petri dish cover
- 4 sheets of paper towel or sphagnum moss
- 1 head of lettuce
- 5 spinach leaves
- 3 20 x 20-cm cheesecloth sections
- 10 g glucose
- 1 100-mL graduated cylinder
- 1 balance
- 2 200-mL beakers
- 1 blender

Optional:

- 1 refrigerator
- 1 tea bag
- 5 aged tree leaves, such as oak, maple, or birch
- 17 mL 58% ammonium hydroxide (NH_4OH)
- 5 mL acetic acid (CH_3COOH) or 100 mL vinegar
- 25 mL 40% ethanol (CH_3CH_2OH)
- 10 g sucrose ($C_{12}H_{22}O_{11}$)
- 10 g sugar substitute
- 10 g salt (NaCl)
- 10 g baking soda

(continued on p. 280)

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Introduce and observe snails for positive or negative response to environmental chemical stimuli. Brainstorm and develop hypothesis.

Day 2 (45 minutes)

- Design and set up experiment.

Day 3 (45 minutes)

- Analyze and interpret the data.

TEACHER'S NOTES

(continued from page 279)

You will need the following for each group of two students in a class of 24:

- ☐ 1 1-L plastic or glass jar with lid or 1 3 x 10 x 10-cm plastic sandwich container with snap-on lid
- ☐ 1 8 x 8-cm piece of 2-cm thick Styrofoam™
- ☐ 1 compass with pencil
- ☐ 2 25-cm filter paper pieces, paper towels, or coffee filters
- ☐ 1 mister/sprayer
- ☐ 1 large land snail (*Helix)*
- ☐ 1 20 x 30-cm plastic sheet protector, report cover, or glass plate
- ☐ 1 25-cm diameter glass pie plate or clear plastic dish
- ☐ 1 permanent marker
- ☐ 1 metric ruler
- ☐ 1 pair of scissors
- ☐ 10 mL spring water
- ☐ 3 25-mL vials
- ☐ 1 forceps
- ☐ 1 clock or watch with second hand
- ☐ 10 mL 10% spinach extract
- ☐ 10 mL 10% glucose solution
- ☐ 1 lab journal

SAFETY PROCEDURES

Use only substances that can be poured safely down the sink to make disposal easier.

Do not use chemicals that are toxic or dangerous to humans.

Never use pure caffeine or nicotine. Avoid pesticides and insecticides.

Check local regulations regarding snails. They may be prohibited in your part of the country, e.g. the transport of snails into Florida and California is prohibited.

Do not release snails into gardens or greenhouses. Both *Helix aspersa* and *Helix pomatia* are European natives that are serious agricultural pests.

Do not permit snails to crawl on lab benches.

Wear safety goggles and aprons when working with chemicals.

Students should wash their hands before and after working with the snails.

Keep hands and fingers away from the compass point when punching holes in lids.

Handle snails with care. Never pull or lift the animal directly from a surface. Rather, gently slide the animal from a surface.

Always maintain a moist environment. Clean container at least once a week. Do not use distilled water or tapwater directly on snails.

DIRECTIONS FOR SETTING UP THE LAB

Preparing and Maintaining the Snail Habitat

- If you use a plastic storage box instead of a terrarium, punch holes into the lid.
- Line the bottom of the terrarium with the sphagnum moss that came with the snails or sheets of paper towel. Fill the jar lid with pond water or aged spring water and place it in the terrarium. To prevent the snails' shells from becoming brittle, place 10 g of calcium carbonate chips or a cuttlebone in the terrarium.

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- Each evening, place a lettuce leaf into the terrarium. Remove any remaining lettuce the following morning.
- The habitat should be placed in a cool location each evening. If possible, place the snails in a refrigerator set to the warmest temperature.
- Replenish the pond water daily.
- Once a week, clean out the habitat by washing the terrarium and jar lid with warm water. Blot the moss to absorb excess moisture and slime, and replace the calcium carbonate.

Preparing the Solutions

Spinach Extract

Puree 2 to 3 spinach leaves in 100 mL of pond or aged water with a blender. Strain the liquid through cheesecloth to remove leaf pieces.

Aged Spring Water

Keep the spring water in an aerated aquarium for a day or more.

10% Glucose, Sucrose, Sugar, Salt, or Baking Soda

Dissolve 10 g of the desired material in a small amount of spring water and dilute to 100 mL with spring water.

Aged Tree Leaves such as Oak, Maple, or Birch (Tannic Acid)

Puree 2 to 3 leaves in 100 mL of water using a blender. Strain the liquid through cheesecloth to remove the leaf pieces.

Tea

Allow 1 tea bag to steep for several minutes in 100 mL boiling water. Cool.

Other substances students may want to test:

10% Ammonium Hydroxide

Add 17 mL of stock (58%) NH_4OH to 83 mL of spring water.

5% Acetic Acid

Add 5 mL acetic acid to 95 mL spring water or use full strength household vinegar.

10% Ethanol

Add 25 mL 40% ethanol to 75 mL spring water.

For lower concentrations of the desired solutions, use the following table.

Table 1. Dilution ratios.

Desired concentration percent	Volume (mL) of 10% solution	Volume (mL) of diluent (spring water)
5	50	50
2	20	80
1	10	90

TEACHER BACKGROUND

Content Information

All organisms must be able to sense the world around them in order to survive. Animals that live in the darkness of caves or the deep sea for their entire lives often have no functional eyes or pigments, but are quite sensitive to chemical stimuli. Bats are sensitive to high frequency sounds that allow them to navigate and find prey in the dark using echo-location. Snakes do not see well, but are able to detect minute amounts of chemicals in the air with specialized "smelling" organs in their mouths. On the other hand, humans have a reduced sensitivity to odors and have come to rely heavily on sight.

Snails are able to sense their surroundings. They need to find food and mates and avoid predators and dangerous situations that not only involve harmful chemicals, but also include potential harm from overheating or dehydration. Land snails, such as *Helix*, sense the environment using two pairs of tentacles. A simple eye is located at the end of each of the longer tentacles. These eyes are able to detect shadows and movement. The shorter pair of tentacles is used for smell and touch. See Figure 1.

PREPARATION TIME REQUIRED

1 hour

- Prepare terrarium for the snails.

30 minutes

- Prepare spinach extract and glucose solution.

TEACHER'S NOTES

When a snail comes in contact with something edible, it will stop and begin eating. The natural food of snails is dependent upon species. If the snail encounters something offensive, it will respond by retracting its tentacles and retreating. Sometimes, a snail even will retract its whole body into its shell. A normal retraction sequence is tentacle invagination, lifting of the anterior part of the foot, folding of that part of the foot along the midline, continued anterior invagination, and finally posterior invagination and withdrawal into the shell. You can use these reactions to test a snail's sensitivity and reaction to various stimuli. See Figure 2.

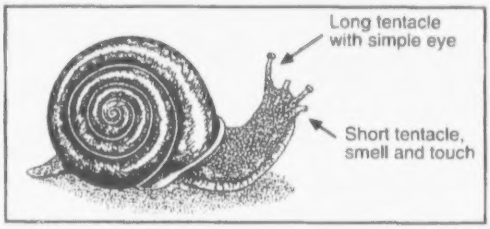

Long tentacle with simple eye
Short tentacle, smell and touch

Figure 1. Snail with tentacles' functions identified.

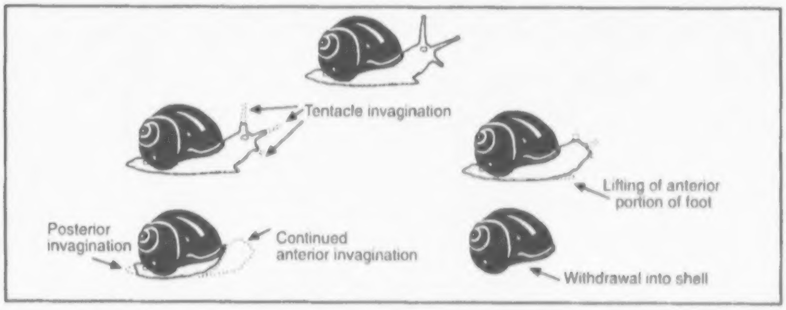

Tentacle invagination
Lifting of anterior portion of foot
Posterior invagination
Continued anterior invagination
Withdrawal into shell

Figure 2. Normal retraction sequence in snails.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

Correct Concept	Misconception
Snails respond to chemical stimuli in their environment.	Snails will not respond to weak chemicals in their environment.
Snails protect themselves from toxic substances by avoiding them.	Snails protect themselves from toxic substances by producing slime.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

For an attention-getting device, microwave a bag of popcorn before class. Allow the aroma to penetrate the room so that it greets the students as they enter the room. If you do not have a microwave, open a bottle of cologne or perfume and allow that aroma to penetrate the room.

Have students list the observations they made as they entered class. Invite them to share their answers. Discuss how humans observe their surroundings using the five senses. Ask the following questions as a lead into hypothesis generation:

- What senses do you use most often?
- Which are the most sensitive?
- Why do you need to be able to sense your surroundings?
- When you taste or smell something, what are you actually sensing?
- Do other animals respond to chemicals in their environment?
- How do you know this?

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

On the board, write a list of up to 10 different animals, including the snail. Include invertebrates as well as some vertebrates. Ask the following questions:

- How does each sense its surroundings?
- Which is the most sensitive to different types of stimuli?
- Which of the listed animals are stimulated by chemicals in their environment?
- What kind of response do they make to chemical stimuli?

At this point, have the students observe snail behavior with a known positive control, such as water, and a known negative control, such as salt solution, so students can define "+" (positive) and "-" (negative) behavior indications. Ask the students to design an experiment to test a snail's response to environmental chemical stimuli. Discuss the importance of testing more than a single animal. Review with your students safe handling of snails and where the snails usually are found so that the hypothesis they suggest has some rational biological basis.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 287.

Question

Do snails respond to different chemical stimuli found in their environment?

Hypothesis

Snails are able to distinguish between various chemicals in their environment.

Rationale

If an animal is not able to detect common hazards in nature, its chance for survival as an adult decreases.

Procedure: *Observation*

1. Wash your hands at the beginning of the lab.
2. Obtain a 1-L plastic or glass container or a 3 x 10 x 10-cm plastic sandwich container with a snap-on lid.
3. Place the lid on a piece of Styrofoam™.
4. Punch holes in the lid with the compass point. Be careful not to put hands or fingers where the compass point could puncture them. See Figure 3.

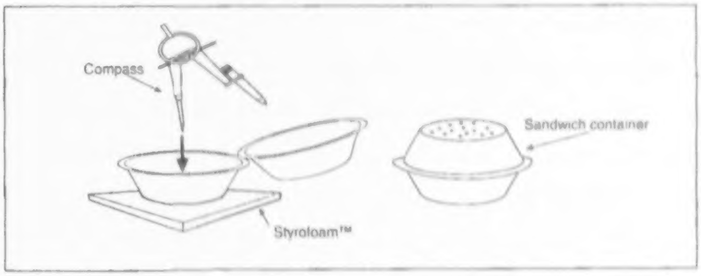


Figure 3. Holes made in sandwich container lid with a compass.

(continued on p. 284)

Sample Hypotheses

- Snails will move toward potential food sources that are green and leafy, but will avoid potential foods with animal products.
- Snails will retract into their shells or will move away from offensive chemicals.

TEACHING TIPS

- Tap water may contain substances that will harm snails. Use pond water or aged spring water in the snails' water dish and in the spray bottles. **Do not use distilled water.**
- The maximum rate of locomotion for the related snail (*Limax maximus*) occurs at about 15°C and decreases at higher temperatures (Jones, 1975). Moving the snails to a warmer location approximately one–half hour before class will stimulate the snails to become more active.
- Before returning the snails to the terrarium at the end of the lab, rinse them in spring water to remove any chemical residues and blot them dry. Place the snails on the calcium carbonate.
- If the experiment takes longer than one class period, have the students label their snail before returning it to the terrarium so that the same snail is used throughout the experiment. Have students dry the snail's shell and label it with a permanent marker, paint pen, or nail polish.
- Students may become very attached to their snails. Remind them that even though they are studying aspects of behavior and senses, there is no evidence that a retreating strategy, or any other behavior, is planned or that the snails have emotions.
- Deciding how far away to set baits may be difficult. You might want to share the following information about *Helix* or other snails summarized from Hyman (1967) with your students or when you guide them in their selection of materials and concentrations. *Note:* Some of these observations require removing tentacles. Although *Pulmonate* snails have a great ability to regenerate almost any part with the exception of the central nervous system, this procedure is not recommended at the high school level. See Table 2, p. 284.

(continued on p. 284)

TEACHING TIPS

(continued from p. 283)

SENSE	OBSERVATIONS
Vision	• Dim perception at 1cm. • Distinct vision at 1.5-2 mm. • Movement of objects does not change perception. • Perceive shadows passing over shell and withdraw.
Touch	• Entire surface is sensitive. • Foot is thigmotactic.

Table 2. Observations of snail senses.

A decrease in response to mechanical shock or vibration, light, and moving shadow has been observed in snails stimulated 20 times (Kerkut & Walker, 1975). Snails recovered in about five minutes and showed no signs of being trained. Students need to allow ample time for snails to recover from stimulation before retesting.

- Snails do not learn well, but students should be able to follow some work on this topic summarized by Hyman (1967).
- If some students are reluctant to handle the snails, they can wear plastic gloves.
- *Helix* hibernates from fall to March or April (Hyman, 1967). It may be difficult to work with them during this period as they estivate when it is dry.
- While your students are becoming acquainted with their snails, have them monitor the amount of lettuce eaten per snail. This background information may be useful for Variation 6.
- According to Behringer (1989), a 40 x 22 x 25-cm (16 x 9 x 10-inch) container is adequate for 25 to 30 snails.
- When ordering the snails, specify snails with shells at least 2.5 cm at the largest dimension.
- Provide a calcium source, such as Tums®, calcium carbonate, or cuttlebones.

(continued from p. 283)

5. Place a folded piece of 25-cm filter paper, paper towel, or coffee filter on the bottom of the container.
6. Mist with spring water to moisten.
7. Obtain snail and place in the container.
8. Observe the snail within the container.
9. Remove the snail from the container and place on a plastic sheet protector, report cover, or glass plate.
10. Observe the snail once more, especially the behavior of the 2 sets of tentacles.
11. Record your observations.

Procedure: Experimentation

1. Mark the bottom of a 25-cm glass pie plate or clear plastic dish into 2 equal sections with a permanent marker. See Figure 4.
2. Fold the 25-cm piece of filter paper in half.
3. Inscribe a circle with a 10-cm radius with the compass as shown in Figure 5.
4. Cut the outer edge of the arc from the filter paper.
5. Cut the remaining piece of filter paper into two equal halves. See Figure 6.
6. Mark each piece with a pencil as follows:
   a. Water
   b. Dry
7. Pour 10 mL of spring water into a 25-mL vial.

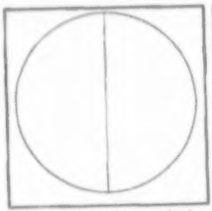


Figure 4. Glass dish divided into two equal halves.

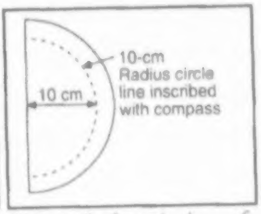


Figure 5. Inscription of circle.

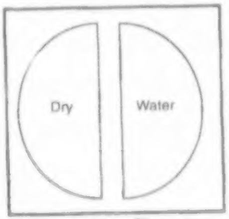


Figure 6. Two filter paper halves.

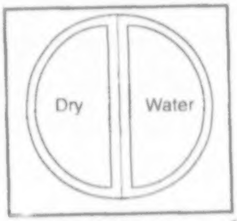


Figure 7. Placement of filter paper halves in dish.

8. Dip one piece of filter paper into spring water with forceps. Leave the other piece dry.
9. Place the filter paper pieces in the dish, equidistant from one another. The pieces of filter paper should not touch. See Figure 7.
10. Place a snail in the center of the dish as shown in Figure 8. Allow it to acclimate to this environment for two minutes.
11. After two minutes, record the number of times that the snail touches each piece of filter paper at 15-second intervals.
12. Continue recording at 15-second intervals for five minutes or 20 counts.
13. Repeat Steps 8 to 12 with spring water. Replace the dry filter paper with spinach extract and then glucose solution.
14. Display your data in a graph and compare them to the results of your classmates.

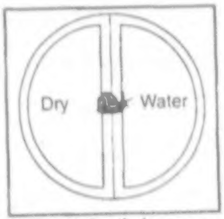


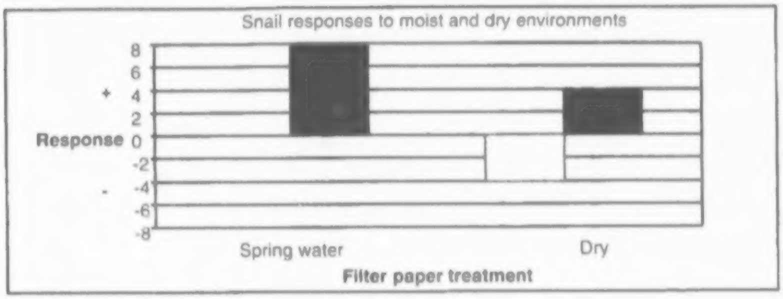
Figure 8. Snail placement in dish.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data 1

Table 3. Responses of snails to moist and dry environments.

Filter paper treatment n=8	Number of snails with negative response	Number of snails with positive response	Comments
Spring water	0	8	Enters
Dry	4	4	Enters or wanders

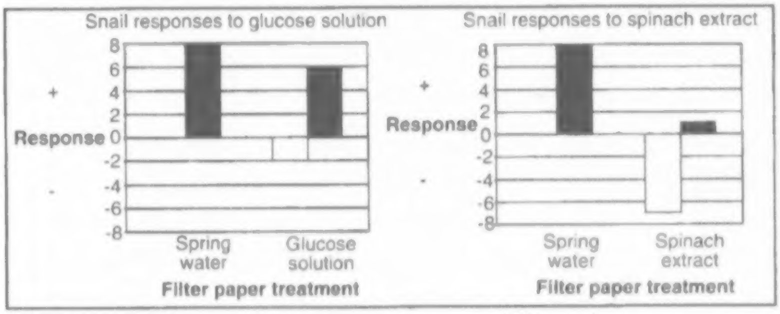


Graph A. Snail responses to moist and dry environments.

Sample Data 2

Table 4. Response of snails to environmental chemicals.

Filter paper treatment n=8	Number of snails with negative response	Number of snails with positive response	Comments
Spring water	0	8	Enters
Spinach extract	7	1	Enters or wanders
Glucose solution	2	6	Enters or wanders

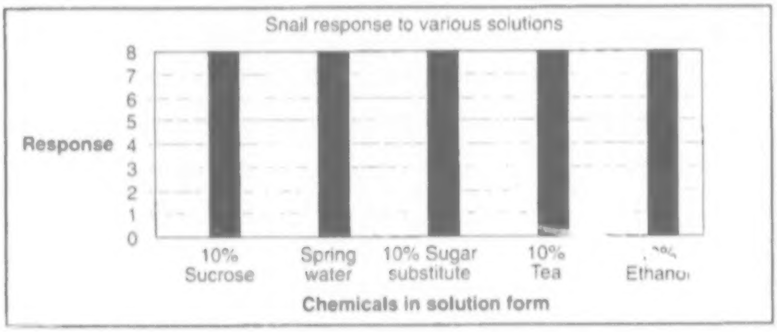


Graphs B1 and B2. Snail responses to environmental chemicals.

Sample Data 3

Table 5. Response of snails to various chemicals

Filter paper treatment n=8	Number of snails with negative response	Number of snails with positive response	Comments
10% Sucrose	0	8	Enters
Spring water	0	8	Enters
10% Sugar substitute	0	8	Enters
10% Tea	0	8	Enters
10% Ethanol	0	8	Enters



Graph C. Snail responses to single concentrations of various chemicals.

Interpretation

These data support the hypothesis. The snails responded to dry and moist environments by moving into or avoiding the dry environment. The snails can distinguish among dry and moist environments. From an evolutionary point, water is a basic requirement of life.

Interpretation

These data do not support the hypothesis. These snails apparently could not distinguish between these chemicals or prefer them equally. All snails moved toward the chemicals and onto the paper with the test solution.

Interpretation

Snails have a positive response to moist environments, as they need moisture to keep sensitive membranes from dehydrating.

Answer to Test Question

A positive response involves movement in the direction of the chemical stimulus; often the foot is stretched as the snail advances. A negative response may be mild, with the snail turning away from the chemical, or strong with the snail lifting its head from the surface before withdrawing into the shell or retreating. We interpreted a lack of movement as a moderately negative response. Snails have a positive response to the control water.

TEST QUESTION

Describe positive and negative response behaviors a snail might exhibit to a particular chemical.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed the data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think about questions that occurred to them as they conducted the Core Experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- What did you observe in your experiment?
- As you were conducting your experiment, what questions came to mind?
- What other factors—chemical, physical, environmental—might influence a snail's behavior? Consider the following:
  -predators
  -food choice and its location
  -effect of light with respect to diurnal versus nocturnal.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students with special needs, who have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to page 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

- Respect the visually impaired or blind student who may or may not be willing to share his/her experience with using taste as a sensory device for the discussion of how humans observe their surroundings. When a sense is absent, it is often said other senses are heightened or more carefully observed.
- Label laboratory shelves of chemicals and labware in braille. The bottles of chemicals and their solutions should be labeled in braille and large print. A strip of sand paper should be attached to hazardous chemicals as a warning. Students who are visually impaired can make minimal observations by touch in an experiment using snails as the primary animal. It will not be possible for them to observe the action of the two sets of tentacles.
- Be aware that students who are visually impaired can make minimal observations by touch in an experiment using snails as the primary animal. It will not be possible for them to observe the action of the two sets of tentacles.
- Provide a copy of the printed laboratory instructions in braille for students who use this reading mode.
- Note that students who are visually impaired should have no difficulty in handling the 25-mm filter paper, placing the halves in a pie plate, and changing solutions as long as materials are accessible.
- Provide accessible, well-labeled solutions so that the student who is blind will have no difficulty in changing the different filter paper sections soaked in various chemicals.
- Assign the laboratory report to be typed by the student who is visually impaired. It should contain the same items that are required of other class members. Bar graphs should be made on braille graph paper. (See "Allelopathy," Variation 1, p. 33).

Deaf or Hard-of-Hearing

- Same considerations as for "Allelopathy," p.29.

Gifted

- One variation that relates well to the garden environment where *Helix* is found is the snail's response to variations in fertilizer concentration.
- Another variation that could bring in skills from physics would be to test the response of *Helix* to additional weight. *Helix* can carry 9 times its weight vertically, but 50 times its weight horizontally (Taylor, 1914 in Jones, 1975).

Manually Impaired

- Be aware that depending on the severity of the impairment, some students will not be able to handle snails or chemical solutions. They can observe the location and behavior of the snails, and report on the position of the tentacles and time actions of snails.
- Assign their laboratory reports to be tape recorded, unless they have a computer with a voice-activated printer. Graphs need to be understood and recalled, rather than drawn.
- Emphasize that the snails should be handled with care. The snails never should be pulled or lifted directly from a dry or wet surface, but be slid along the surface until they move readily.
- Use a light probe to locate and follow the snail in the dish.

Mobility impaired

- Same considerations as for "Allelopathy," p. 30.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment may also include the following:

1. List the chemicals that elicited a negative response in the snail.
2. Ants are able to travel great distances from their nest to obtain food. They have an unerring ability to always return to their nest if their trail has not been disturbed. This indicates the ant's ability to:
   A. detect gravity.
   B. use the sun as a guide.
   C. follow chemical stimuli.
   D. use magnetic fields as a guide.

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Answers to Additional Test Questions

1. Answers will vary depending on class observations. A possible list may include: very strong salt, alcohol, ammonia, and/or vinegar.
2. C

Answers to Questions and Analysis on Student Page

1. These graphs should resemble Graphs B1, B2, and C.
2. The answer to this question will depend on the data. If the snails consistently avoid some material and move toward others, accept the hypothesis. If the snails go to every choice in approximately equal numbers, reject the hypothesis.
3. The previous recent experience of the snails might influence their preference; they may prefer a familiar substance. Hunger may influence its selection; a snail that has recently eaten may be more selective than one that is well fed. The choices offered might influence a snail's choice. If it has to choose between two things it does not like, it still may choose something it does not like. It would be useful to know whether any "no choice" options are provided for the snail; that is, are there places it can go simply to escape being tested? Sampling error also could produce the differences seen. It would be useful to know the number of animals that were tested in each experiment.
4. Knowing the sample size certainly could influence confidence in the conclusion. If 4 of 4 animals made one choice, that would be 100% of the animals tested, but the sample size is so small that chance could account for the results. If 24 of 30 snails make one choice, chance would be accepted less likely as an explanation. It could be concluded that these snails have a preference.
5. To test whether odor influences the movement of a snail toward the material, disguise some frequently selected material and treat it with an aromatic material. To focus the source of the odor, put the snail in the middle of a cylinder and put a choice at each end of the cylinder. Test several snails that exhibit an attraction toward the untreated material.

BEST COPY AVAILABLE

TEACHER'S NOTES

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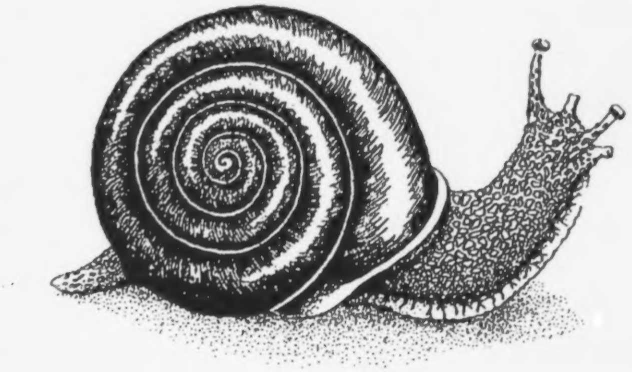
POSSIBLE SOURCES OF MENTORS

United States Department of Agriculture, Beltsville, MD

Variations on the Core Experiment

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.



VARIATION 1

VARIATION 1

The Effect of Spinach Extract and Glucose Dilutions on Snail Response

SYNOPSIS

Students will determine if changing the concentration of the spinach extract solutions will affect the snail's response to them.

ADDITIONAL MATERIAL NEEDED

You will need the following for each group of two students in a class of 24:

- 1 calibrated dropping pipette
- 1 5-cm vial
- 0.5 mL spinach extract
- 0.5 mL 10% glucose solution

HYPOTHESIS GENERATION

Question

How will changing the solution's concentration affect the snail's response?

Sample Hypothesis

Changing the concentration of the chemical solutions will not change the snail's responses.

Rationale

You expect that at some low concentration most chemicals will not be harmful and the ability to detect them should be low. Above the first level of detection, you might expect no change in the intensity of the response. If a snail is starving, it may consume some materials in excess that can be harmful. So, you may find exceptions. You may reject or accept a hypothesis in this form if you choose to do a statistical analysis. See Variation 4 for directions on statistical analysis.

Sample Experimental Procedure

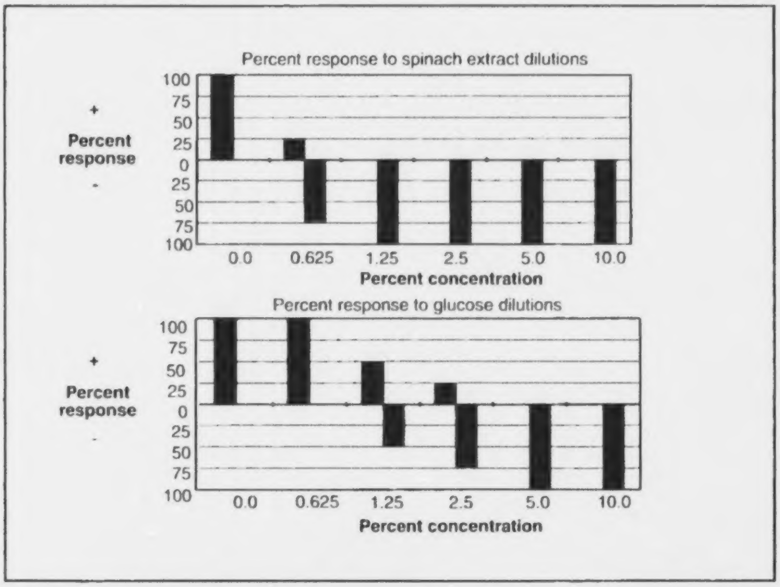
Repeat the Core Experiment procedure, but alter the concentrations of the solutions.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Responses of snails to various concentrations of chemicals used in the Core Experiment are shown above. Reported values are number of snails showing a positive response in the numerator and number of snails showing a negative response in the denominator.

	Percent response at various concentrations					
Chemical	0	0.625	1.25	2.5	5.0	10.0
Spinach extract dilutions	4/0	1/3	0/4	0/4	0/4	0/4
Glucose dilutions	4/0	4/0	2/2	1/3	0/4	0/4



Graphs D1 and D2. Snail responses to varying concentrations of spinach extract and glucose solution.

TEACHING TIPS

- Use lower concentrations of the original tested solutions if the response was negative; use higher concentrations if the original response was positive.
- Provide solutions or instructions and pipettes for making solutions of varied concentrations of spinach extract-glucose solutions, such as 1.5, 2.5, 5.0, and 10%. Make the differences 50% to an order of magnitude. You may extend the range of concentrations tested by providing a more concentrated solution as well.
- This is a good place to explain how to make dilutions and have students prepare their own solutions. To make the dilutions used to generate the sample data, use a calibrated dropping pipette to add 0.5 mL of a solution into a 5-cm vial. Then, add 0.5 mL of spring water. The next dilution is made by transferring 0.5 mL of that dilution into another small vial and adding 0.5 mL of spring water. Each solution is half the concentration of the preceding solution. See Figure 9.

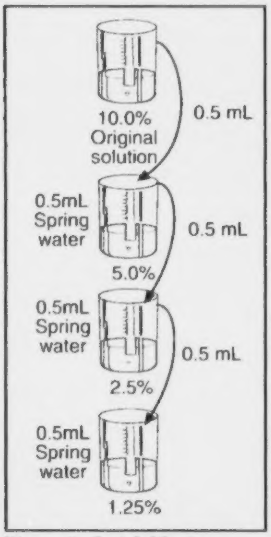


Figure 9. Serial dilution.

(continued on p. 290)

Interpretation

Reject the hypothesis if the snails show negative responses to some concentrations and positive responses to other concentrations. Accept the hypothesis if the snails show the same response to all concentrations of the chemicals.

TEST QUESTION

Study Table 7 below and answer the following questions. Are snails able to detect different concentrations of chemicals? What evidence supports your answer?

Table 7. Responses of snails to various concentrations of chemicals. As in Table 6, reported values are number of snails showing a positive response in the numerator and number of snails showing a negative response in the denominator.

Chemical	Percent response at various concentrations					
	0	0.625	1.25	2.5	5.0	10.0
Ammonium hydroxide	4/4	1/3	0/4	0/4	0/4	0/4
Ethanol	4/0	4/0	2/2	1/3	0/4	0/4

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Deaf or Hard-of-Hearing

- Prepare solutions as done in "Allelopathy," Variation 1, p. 33 if not prepared by the instructor.

VARIATION 2

The Effect of Gravity on Snail Behavior

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will determine whether snails are positively or negatively geotactic.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 1 10 x 10-cm glass plate
- 1 protractor

HYPOTHESIS GENERATION

Question

What type of geotactic response do snails exhibit?

Sample Hypothesis

Snails will exhibit a negative geotactic response.

Rationale

Helix eggs are laid below ground, and the hatchlings need to reach food above ground. Snails are primarily active at night when light is not available, but gravity is. Movement in the direction opposite the force of gravity is a negative geotactic behavior.

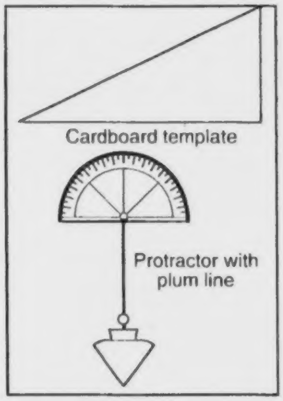


Figure 10. Mechanisms to determine inclination angle.

Sample Experimental Procedure

1. Set a snail at the center of a glass plate in an orientation parallel to the ground. Mist the glass lightly with spring water.
2. Slide the glass to the edge of the lab bench. Position the protractor or inclination angle template against the edge of the plate at the snail's posterior.
3. Gently tilt the plate upward to the desired angle. Measure the angle of inclination as a deviation from the true horizontal as shown in Figures 11a and 11b.

Answer to Test Question

Snails are able to detect differences in concentrations of the chemicals tested. They responded negatively to concentrations of ammonium hydroxide greater than 0.625%, but did not exhibit a negative response to lower concentrations. They also responded negatively to concentrations of alcohol that were equal to or higher than 1.25%.

TEACHING TIPS

(continued from page 289)

- Some species of *Helix* eliminate nitrogenous waste, such as ammonia (Machin, 1975). You might expect that they will show some tolerance for low concentrations of ammonia.
- If the test chemical has a strong odor, begin tests with the lower concentrations and keep the concentrated solutions covered on a distant lab bench. Consider treating everything as though it has a strong odor, since you have not determined whether a snail's olfactory acuity is similar to ours.
- Analyze results as percent responding in a particular way to make data clear when sample sizes are not the same.
- You can cut cardboard sections at the desired inclination angle and substitute these as templates for the protractor with plumb line. A string and weight may be attached to the protractor to act as a plumb line. See Figure 10.
- Snails have two small, liquid-filled statocysts approximately 200 µm in diameter. When the animal is upside down, the statoliths stimulate hair cells in the equilibrium organs called statocysts. These organs are not stimulated in other positions (Kerkut & Walker, 1975).
- Snails climb upward over the range of 15° to 75°. Within this range, they can detect a difference of 2°. If the climbing plane is rotated, so that the snail becomes briefly horizontal, the time needed for reorientation depends on the angle of inclination. At 90°, 2.88 seconds are needed; but at 5°, 12.15 seconds are needed (Hyman, 1967).
- You can substitute plastic for the glass plate, but snails adhere better to the glass.
- Modeling clay can be used to anchor the plate glass at the desired angle.

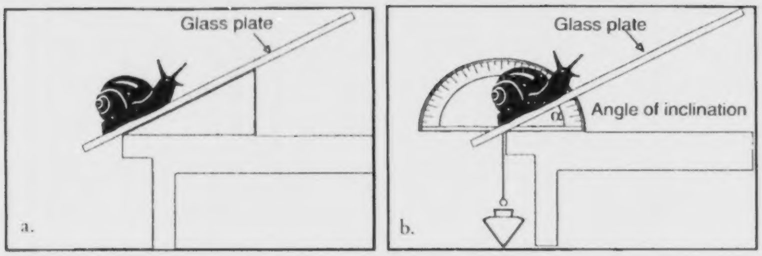


Figure 11a. Setup with cardboard template for angle of inclination. **Figure 11b.** Setup with protractor and plumb line for angle of inclination.

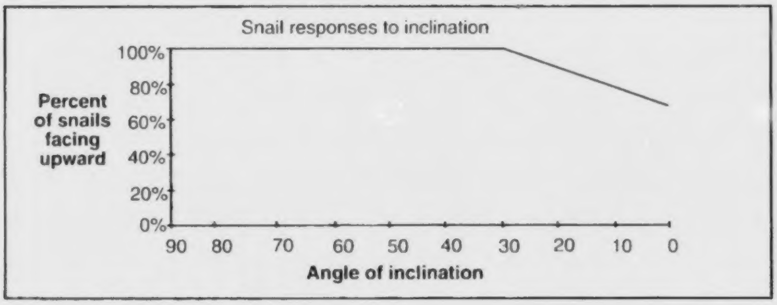
4. As soon as the snail has moved clearly to reorient its head, record whether the new orientation is head up or down. Then, return the glass plate to a horizontal orientation.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 8. Responses of snails to inclination.

Angle (°) of inclination	Number of snails facing upward	Number of snails facing downward
90	8	0
45	8	0
22	8	0
11	8	0
5	6	2



Graph E. Snail responses to inclination.

TEST QUESTION

Why do you usually find the snails you placed on the bottom of the terrarium clinging to the cover in the morning?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have visually impaired students participate as a part of a group in this experiment. Some may have or can acquire a braille protractor that can have a plum line attached. If so, they should be able to determine the angle of inclination.
- Tape the edges of the glass plate if it substituted for a plastic plate.
- Collect data in braille for use in the lab report.
- Provide commercial models of snails, if they are available. Lab partners, lab assistants, and friends are often willing to make models if they are aware of the need. They also may produce raised-line drawings and braille paintings.

Interpretation

Reject the hypothesis if the snails climb upward more often than downward or do not climb in any particular direction. Accept the hypothesis if the snails climb downward more often than upward.

Answer to Test Question

Snails that are active during the night can sense up and down. They move upward most often during experimentation. The underside of a leaf is usually more tender than the upper surface where a thicker cuticle protects against water loss. The tender underside would be easier for the snail to eat than the upper surface. If a snail is on the under surface of a leaf, it is probably well hidden from predatory birds. Off the forest floor, it also may be somewhat protected from predatory mice.

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TEACHING TIPS

- *Helix aspersa* is negatively phototactic, moving away from a light source (Kerkut and Walker, 1975). The light intensities demonstrated in this reaction are not specified. The direction that light falls on the photoreceptors—sense organs that respond to light—at the ends of the dorsal tentacles, may be important in determining the response. There are no other photoreceptors on *Helix*.
- Most snails and slugs are crepuscular, preferring the low light of dusk, or nocturnal (Cain, 1983). Sometimes, *Helix pomatia* gather in a lighted area (Hyman, 1967). The greatest time of activity is from midnight to 3 a.m. Some cave-dwelling snails show similar diurnal rhythms. You may find different activity levels in an 8 a.m. class and a 1 p.m. class.
- You can create a heat trap by putting a clear, colorless cake pan of cold water between the light source and the snail tubes.
- You can expand this variation by using layers of screening or gray report covers to create a series of increasingly dark regions along a tube. Use a camera light meter to quantify the amount of light under each treatment. You might want to randomize the arrangement of the treatments or use a pair-wise arrangement, such as the design used in the next variation.

VARIATION 3

The Effect of Light on Snail Behavior

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will determine if snails show positive or negative phototactic behavior.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two to four students in a class of 24:

- 1 lamp, light bank, or bright window not in direct sunlight
- 1 15 x 15-cm piece of clear, colorless transparency film or report cover
- 1 15 x 7.5-cm piece of black construction paper or opaque black plastic
- 2 rubber bands

HYPOTHESIS GENERATION

Question

What form of phototactic response do snails exhibit?

Sample Hypothesis

Snails will exhibit a negative phototactic response.

Rationale

Snails are active mostly at night. During daylight, it is usually warmer and drier. The loss of water required to remain cool in the light and warmth of day could be great. Movement away from light is a negative phototactic behavior.

Sample Experimental Procedure

1. Use a permanent marker and ruler to draw a line across the middle of the transparency film. Lay the marked side down to avoid any problems with potentially toxic ink.
2. Place your snail on the line orienting the body along the line.
3. Lightly mist the transparency film with spring water. See Figure 12, Step 1.
4. Roll the transparency film into a tube about 4 to 5 cm in diameter and fasten it with a rubber band. See Figure 12, Step 2.
5. Quickly roll black construction paper or opaque plastic over one half of the tube and fasten it with a rubber band. See Figure 12, Step 3.

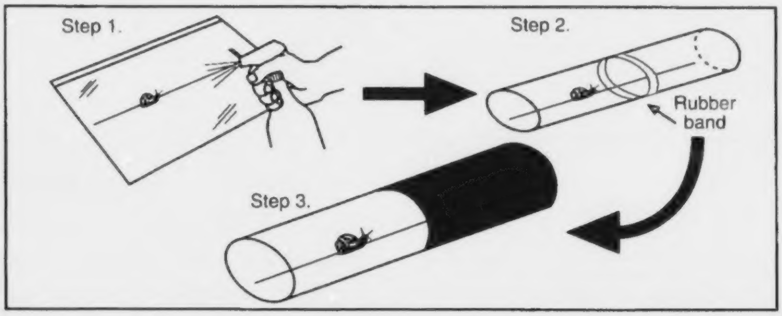


Figure 12. Procedure steps.

6. Lay the tube under the lights. Do not put it close to an incandescent bulb because the temperature can become dangerously high for the snail.
7. Observe the snail's behavior at 1-minute intervals for 10 minutes.
8. Record the direction of the snail's response as "+" if it moves toward the bright half of the tube or as "-" if it moves toward the dark half of the tube.
9. At the end of 10 minutes, disassemble the setup and return the snail to its original container.
10. Use a damp paper towel to clean slime from the snail side of the transparency film and repeat your observations with another snail.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 9. The phototactic behavior of snails.

	Positive response	Negative response
Number of snails	0	10

Data as overwhelmingly clear as these require no further analysis. If you want to create a graph, use a bar graph with light treatment on the x-axis and number of snails or percent of responding snails on the y-axis.

TEST QUESTION

What time of day do you expect snails to be active? How do the results of this experiment support your answer?

SUGGESTED MODIFICATION FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide a counter or place for a table underneath a window, if available. It is a better place for the student who is blind to place his/her tubes. The student who is visually impaired then can use a light sensor to follow the location of the snail in the tube.

VARIATION 4

The Effect of the Color of Light on Snail Movement

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS

Students will determine if snails prefer light of a particular color.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 1 lamp or bright window, not in direct sunlight
- 1 15 x 15-cm piece of clear, colorless transparency film or report cover
- several 15 x 7.5-cm pieces of cellophane, colored transparency film, or report covers
- 3 rubber bands

HYPOTHESIS GENERATION

Question

Are snails able to detect different colors?

Sample Hypothesis

Snails will show no preference for light of a particular color.

Rationale

Although it is not likely that an animal which is active at night is able to discern color, it is possible that it can detect differences in wavelength characteristic of dawn and twilight.

Sample Experimental Procedure

1. Determine the number of sheets of each colored transparency required to transmit equal light intensities as follows:
   A. Arrange the light meter so that the needle is not deflected full scale.
   B. Set a single sheet of the darkest color over the sensor and record the result.
   C. Remove that sheet and replace it with a single sheet of another color. Add more sheets of the less light-blocking color until the meter reading is the same as the value you recorded for the single darker sheet. Use the same number of sheets of the second color in your experiment.

(continued on p. 294)

Interpretation

Reject the hypothesis if the snail consistently moves toward the light or shows no definite directional preference. Accept the hypothesis if the snail consistently moves away from the light source.

Answer to Test Question

The snails all moved away from the light, so they probably are active at night. A range of light intensities was not tested. They may move from very bright light to less light such as from the top of a leaf in direct sunlight to the bottom of a leaf, and actually be active during the day. An extension of this experiment would be to test snail responses to a range of light intensities.

TEACHING TIPS

- Once your students have rolled the colored transparency films to the correct size and fastened them with rubber bands, they may be able to slip them on and off the colorless tube for quick setup. Alternatively, you may want to provide tape for fastening the films.
- Remember that if you want to do a Chi-Square Goodness-of-Fit, you must have expected values of at least five for each snail.
- This setup is more a technique that detects unsatisfactory light environments rather than preferred light environments. It is a good starting point that can lead to some interesting discussions.
- Keep a record of the snail responses in the design grid, then summarize the data as shown in Sample Data of this variation.
- Encourage your students to graph their data with the colors arranged by increasing wavelength: violet-blue-green-yellow-orange-red.
- Transparent colored films, such as cellophane or report covers, are not spectrally pure. This will not invalidate the results if you state them in terms of the materials used rather than specific wavelengths.
- The intensity of light transmitted by a single sheet of different colors will be different. To avoid complicating the experiment, adjust the number of sheets you use to create the different colors to pass light of approximately the same intensity. Your adjustment will be approximate because you can make only whole-sheet adjustments.
- An inexpensive plant light meter

(Continued on p. 295)

Interpretation

If snails do not exhibit color preference, we may not assume that they do not have color vision; only that over the range tested, they had no preference. Reject the hypothesis if any color attracts more snails than expected by chance alone. The color with the most snails is the preferred color. Accept the hypothesis if an equal number of snails moved toward each color.

(continued from p. 293)

D. Continue with this technique until you have determined the number of sheets needed for each color to transmit equal light intensities.

2. Then, repeat Steps 1 to 4 of the Sample Experimental Procedure in Variation 3.
3. Quickly roll colored transparent film, instead of black construction paper, over one half of the tube and fasten it with a rubber band. See Figure 13.

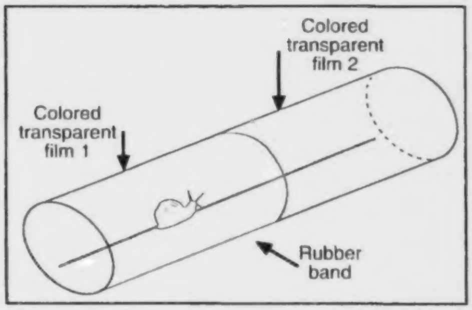


Figure 13. Cover the transparency with colored transparent film.

4. Cover the other half of the tube in the same manner, but use a different color. Table 10 shows the arrangement of colors you should create.

Table 10. A grid of color combinations providing an equal number of chances to choose each color.

	Color A	Color B	Color C	Color D	Color E
Color A	XXX	AB	AC	AD	AE
Color B	XXX	XXX	BC	BD	BE
Color C	XXX	XXX	XXX	CD	CE
Color D	XXX	XXX	XXX	XXX	DE
Color E	XXX	XXX	XXX	XXX	XXX

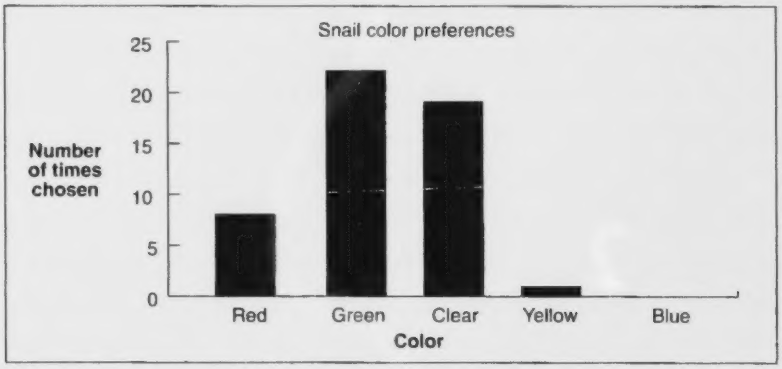
5. Lay the tube under the lights. Do not put it close to an incandescent bulb because the temperature can become dangerously high for the snail.
6. Record the color the snail selected at 1-minute intervals over a 10-minute time period.
7. Use a damp paper towel to clean slime from the snail side of the transparency film and repeat your observations with another snail.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 11. Snail color preferences.

n=10	Red	Green	Clear	Yellow	Blue
Number of times chosen	8	22	19	1	0



Graph F. Snail color preferences.

Analyze the data statistically with Chi-Square Goodness-of-Fit. To perform the statistical test:

1. *Determine expected frequencies*: You expect a particular relationship in your results. The relationship in this example is the same number of individuals in each treatment or 50/5 = 10. That is, the total number of observations divided by the ways they can be observed.
2. *Calculate Chi-Square*: For each color, subtract the expected value from the observed value, square the difference, and divide by the expected value. In equation form, this is:

$$\sum = \frac{(\text{Observed - Expected})^2}{\text{Expected}}$$

   Add all of the quotients to obtain a Chi-Square value.
3. *Compare*: In row (v) of the table, use one less than the number of classes. In this experiment, we have used five classes. Compare your calculated Chi-Square with the values in the table for $v = 4$. For $\alpha = 0.05$, Table 12 shows the expected Chi-Square values.
4. *Conclude*: If your calculated value is greater than the value in the table, you may conclude that the difference between your results and those hypothesized is not likely due to chance alone. You, therefore, reject the null hypothesis. With an $\alpha = 0.05$, 5% of the time you may reject a hypothesis of no difference when a difference exists.

TEST QUESTION

Could color play a role in food location by your snails?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Use a light sensor to compare transmission of equal light intensities through sheets of colored transparent material. In order to test more than one color at a time and to have better conditions for using the light probe to follow the travels of the snail, a simple device is suggested.
- Place strips of colored transparent material on the sides and lid of a clear, plastic shoe box 30 to 35 cm (12 to 14 inches). Drape a black cloth over the end.
- The lid on the box can be removed easily to use the light sensor. If the lid that comes with the box is a solid color, substitute a clear piece of plastic for the cover.
- See Figure 14.

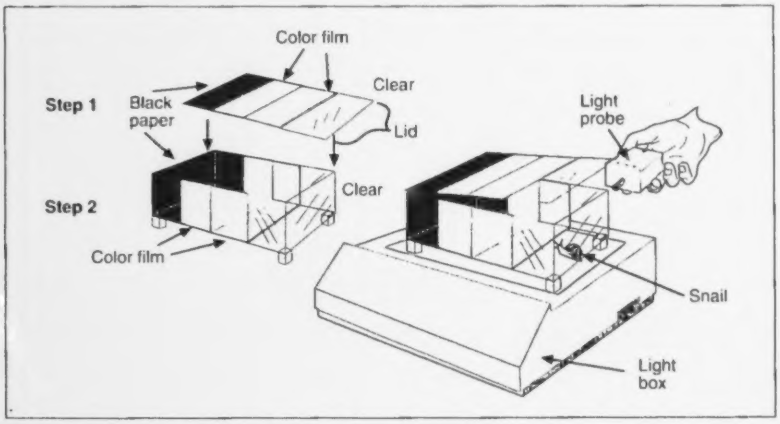


Figure 14. Simple device for testing the effects of more than one color at a time

Deaf or Hard-of-Hearing

- Be aware that Chi-Square may be new to most of the students and may be difficult to understand for some students who are visually or hearing impaired. This may require the use of a magnetic board and large magnetic numbers. The assistance of math teachers and mature lab assistants may be beneficial.

TEACHING TIPS

(continued from p. 294)

with a linear scale should be adequate to measure the light transmitted. Biological supply companies, discount mail-order tool catalogs, and nurseries usually sell these devices. You can make a light meter with the solar cell from an inexpensive calculator and a voltmeter. You may be able to borrow a voltmeter from the physics or electronics instructor or from the maintenance shop.

- If a window with good light is not available, an electric viewing box, such as that used to sort 35 mm slides, is suggested. The school's Audio Visual (AV) Department may have a 35mm viewing box.

Table 12. Expected Chi-Square values for $\alpha = 0.05$.

v	Expected Chi-Square value
1	0.455
2	1.386
3	2.366
4	3.357
5	4.351
6	5.348

Answer to Test Question

If snails showed a strong preference for particular colors commonly found in plant leaves and fruits, these colors may help the snail locate food.

TEACHING TIPS

- Heat transfer will be better through the transparency film than through the glass plate.
- Meat or pastry trays are good sources of Styrofoam™ for insulation.
- You may want to modify the design by placing four bags together and setting the snail at the intersection of the bags. See Figure 15. If you use this modification, select only four temperatures to test. This modification will require fewer replicates.

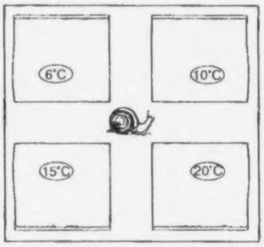


Figure 15. Setup with four bags at varying temperatures.

- The movement of poikilotherms, including snails, is influenced strongly by temperature. *Limax* is a slug with only a rudimentary shell. For *Limax maximus*, both the speed that a muscle wave moves along the foot and the step length (the distance a point on the foot moves as the wave passes over it) affect the rate that the snail moves. An increase in temperature increases the wave frequency, but decreases the step length. Over the temperature range of 6 to 30°C for this species, the rate of movement increases to 15°C, then decreases as the temperature increases (Jones, 1975).
- To have expected values of at least five for each cell, remember Chi-Square Goodness-of-Fit to determine the number of trials and the number of temperatures to test.
- This experiment is more a technique that detects unsatisfactory temperature environments rather than preferred temperature environments. It is a good starting point for interesting discussions.
- Keep a record of the temperature choices using the original design grid. Summarize the data as presented in the Sample Data. Refer to the original data record as well as to the summary when writing the conclusions and discussion.

VARIATION 5

The Effect of Temperature on Snail Behavior

Note to Teachers: *In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.*

SYNOPSIS

Students will determine if snails show positive or negative thermotactic behavior.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 2 zip-closure, plastic sandwich bags
- 1 centigrade thermometer
- 2 600-mL beakers or 16-oz jars
- ice
- 600 mL tap water
- 1 Styrofoam™ meat or pastry tray
- 1 15 x 15-cm piece of transparency film or report cover

HYPOTHESIS GENERATION

Question

Do snails have a temperature preference?

Sample Hypothesis

Snails will have no temperature preference.

Rationale

The phrasing is designed to use statistical analysis. One would expect to find some temperature preference. You might even expect that preference to be narrow in snails who regulate their body temperature behaviorally, rather than metabolically.

Sample Experimental Procedure

1. Use ice and tap water to create two beakers of 300 mL of water at two different temperatures. Temperatures of 5 to 30°C should be safe for the snail. Table 13 shows a possible range of temperatures.
2. Put the water from each beaker into a sandwich bag. Express the excess air and seal the bag.
3. Put the bags next to one another and set your glass plate or transparency on top of them. See Figure 16.
4. Put your snail on the glass plate or transparency so that its body is at the juncture of the bags and parallel to it.
5. Lightly mist the plate or transparency and allow the snail time to move and record the temperature it selects.
6. Use a damp paper towel to clean slime from the snail side of the glass plate or transparency film, and repeat your observations with another snail.

Table 13. A grid of temperature combinations providing an equal number of chances to choose each temperature.

	50°C	40°C	25°C	10°C	0°C
50°C	XXX	AB	AC	AD	AE
40°C	XXX	XXX	BC	BD	BE
25°C	XXX	XXX	XXX	CD	CE
10°C	XXX	XXX	XXX	XXX	DE
0°C	XXX	XXX	XXX	XXX	XXX

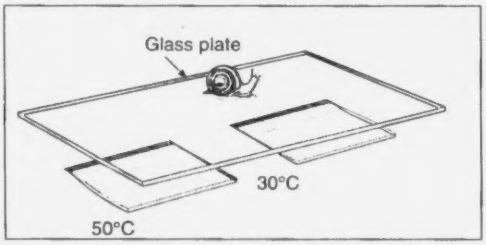


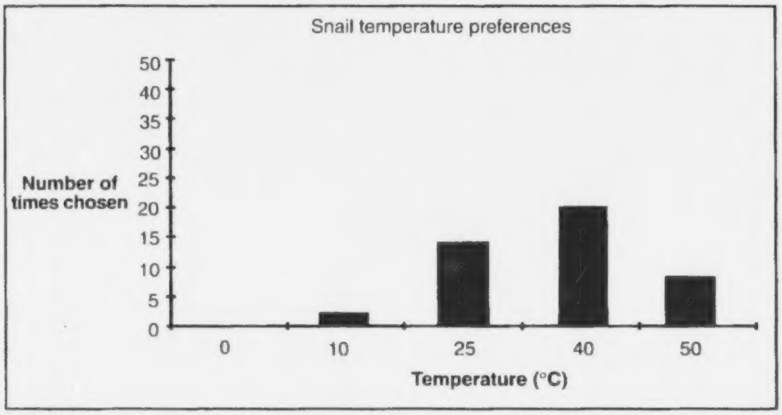
Figure 16. Setup for experiment.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 14. Snail temperature preferences.

	50°C	40°C	25°C	10°C	0°C
Number of times chosen	8	17	14	1	0



Graph G. Effect of temperature on snail behavior.

TEST QUESTION

Explain why snails are most active at night or on cloudy days, but not active during cold winters.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- This experiment requires no new laboratory techniques for the students who are visually impaired. They may wish to use the Talking Thermometer to check the temperature on top of the glass plate in various locations.
- See Considerations for Variation 4 for Deaf or Hard-of-Hearing.

VARIATION 6

The Effect of Plant Food Choice on Snail Feeding Behavior

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will determine if snails have a preference for a specific plant food.

ADDITIONAL MATERIAL NEEDED

You will need the following for each group of three students in a class of 24:

- pieces of several different types of food, including apples, lettuce, spinach, broccoli, and carrots.
- 1 balance *(optional)*
- 1 blender or mortar and pestle *(optional)*

HYPOTHESIS GENERATION

Question

Do snails avoid food that is toxic?

Sample Hypothesis

When offered a choice of fresh produce, snails will show no preference.

Interpretation

Reject the hypothesis if any temperature has more snails than expected by chance alone. The temperature with the most snails is the preferred temperature. Accept the hypothesis if an equal number of snails moved into each area.

Answer to Test Question

The snails never chose the highest temperature offered. They were most active at cooler temperatures like those that occur at night during the summer or on cloudy days. Even if they could find food on a very cold winter day, they only chose the coldest temperature when the other choice was a high temperature.

Rationale

Snails must be able to distinguish between foods that are toxic and those that are safe.

Sample Experimental Procedure

1. Prepare the filter paper strips with extracts of the various foods as in the Core Experiment.
2. Place the strips of paper in a circle on the plastic sheet protector leaving an equal space between each piece. See Figure 17.
3. Lightly mist or dampen the plastic inside the circle.

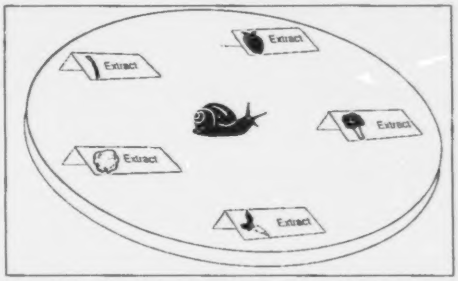


Figure 17. Arrangement of plant food choices.

4. Place one snail in the middle of the circle.
5. Observe and record which direction it travels and how it reacts when it reaches each strip.
6. If the snail does not move after it has reached a strip, pick it up carefully and return it to the middle so it is facing another direction.
7. Repeat Steps 4 to 6 four times.

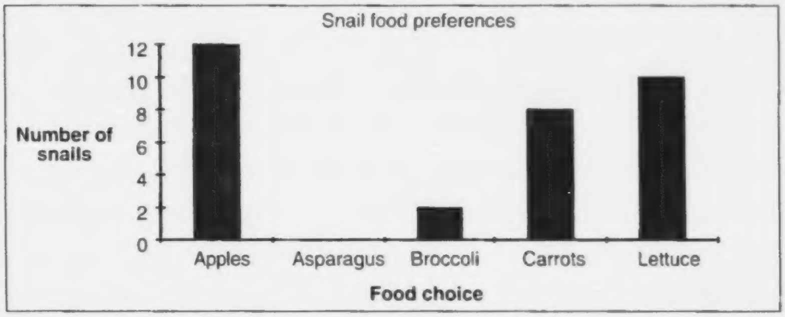
SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 15. Snail food preferences.

	Food choice				
	Apples	**Asparagus**	**Broccoli**	**Carrots**	**Lettuce**
Number of snails selecting the food	12	0	2	8	10

If you want to do a statistical analysis, Chi-Square Goodness-of-Fit is appropriate (see Variation 4).



Graph H. Snail food preferences.

TEST QUESTION

If you were keeping snails in a terrarium, what would you feed them to be certain that they had the essential nutrients for growth and health?

TEACHING TIPS

- The snails may not respond well to this design if they are not hungry. Students may want to do this as an extended exercise, collecting information on two foods at a time and overnight. If a snail does not eat a particular food, it may be that the uneaten food was not encountered at all or was encountered when the snail was no longer hungry.
- If students have fed the snails lettuce during the introductory period of this lab and recorded the amount eaten, they will have some idea of how much a single snail will eat. They can then offer two different foods, each in half the amount that a snail would normally eat. They could offer four foods at once, each in one-fourth the amount of the total food normally consumed, and so on.
- A good way to measure the amount eaten is to mass the fresh food when it is offered and again when it is removed. A separate container with wet paper towel and food pieces the same size as those offered can be used to adjust the final mass for loss by evaporation.
- A third possible design for this variation is to use the puree without the paper.
- In the field, snails are selective in their diet and may not eat from all their preferred plants in an area (Runham, 1975). They may have short term preferences, eating one type of food exclusively for several days. The maturity of the plant material may alter its palatability.
- Juvenile and mature snails differ in their food preferences (Runham, 1975).

Interpretation

Reject the hypothesis if any food is selected more or less often than the others. Accept the hypothesis if an equal number of snails select each food choice.

Answer to Test Question

Although broccoli and asparagus are nutritious for humans, they would not be good choices to feed snails. Snails do not eat them. Terrarium snails would be fed a mixture of fresh vegetables including apples, lettuce, and carrots. These foods were most often chosen by the experimental animals.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Notch the edge of the large filter paper to identify the food at each location. The samples may be placed on a large circle of wax paper taped to the lab table. Some students who are visually impaired will be able to identify the pureed foods by smell.

VARIATION 7

The Effect of Bacterial Decomposition on Food Preference by Snails

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students will determine if fresh or decaying lettuce is preferable to snails.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- decayed lettuce
- fresh lettuce

HYPOTHESIS GENERATION

Question

Do snails prefer fresh or decayed food?

Sample Hypothesis

Snails will prefer fresh lettuce over decaying lettuce.

Rationale

Creation of silage makes more protein available to livestock. Spoilage of the lettuce similarly may make different nutrients available to the snails.

Sample Experimental Procedure

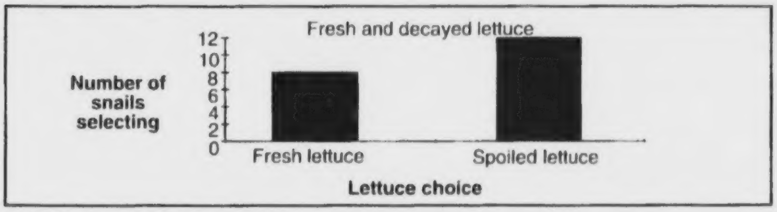
1. Prepare the separate filter paper halves with purees of the fresh or decayed lettuce and place them in the glass dish as shown in the Core Experiment.
2. Lightly mist or dampen the plastic between the filter paper halves.
3. Place 1 snail between the filter paper halves as shown in the Core Experiment.
4. Observe and record which direction the snail moves and how it reacts when it reaches each half.
5. If the snail does not move after it has reached a half, pick the snail up carefully, return it to the center of the dish, and face it in the other direction.
6. Repeat Steps 3 to 5 four times.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 16. Snail food preferences.

	Food choice	
	Fresh lettuce	Decayed lettuce
Number of snails selecting the food	8	12



Graph 1. Snail food preferences.

TEACHING TIPS

- To decay the lettuce slightly, store it wet in plastic in the refrigerator for several days to encourage bacterial growth.
- Students may volunteer to bring lettuce from home.
- Several snails have been shown to prefer decaying vegetation to fresh vegetation in the field (Runham, 1975).

Interpretation

Reject the hypothesis if the same number of snails select each choice, or if more snails select the decaying lettuce than the fresh lettuce. Accept the hypothesis if more snails choose fresh lettuce.

TEST QUESTION

Would you predict that more crop damage will occur in a small garden plot where plants are heavily mulched with waste lettuce leaves or in a plot with the same distribution and kinds of plants but with a mulch of nutrient-poor straw? Explain your prediction.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

- See Considerations for Variation 6.

VARIATION 8

The Effect of Plant Versus Animal Food Choice by Snails

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will determine whether *Helix* prefers animal or plant material for food.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- a selection of animal materials such as dead snails, sections of dead earthworms, hamburger, or other supermarket raw meats

HYPOTHESIS GENERATION

Question

Will snails prefer plant material over animal material?

Sample Hypothesis

Snails will prefer plant material when offered a choice of plant or animal material.

Rationale

Helix is such a serious agricultural pest that there are restrictions on importing it into some states.

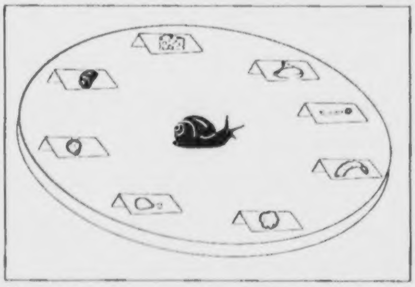


Figure 18. Arrangement of plant and animal food choices.

Sample Experimental Procedure

1. Arrange plant and animal food choices in a circle on the glass or plastic experimental surface leaving an equal space between each choice. Be certain to provide an equal number of plant and animal choices.
2. Lightly mash each food choice to simulate damage that might occur in the field.
3. Lightly mist or dampen the plastic inside the circle.
4. Place one snail in the middle of the circle.
5. Observe and record which direction it travels and how it reacts when it reaches each food choice.
6. If the snail does not move after it has reached a choice, pick it up carefully and return it to the middle so it is facing another direction.
7. Repeat Steps 4 to 6 several times.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 17. Snail preferences from plant and animal food choices.

	Food choice			
	Lettuce 1	Lettuce 2	Snail	Grasshopper
Number of snails choosing a particular food source	2	3	15	10

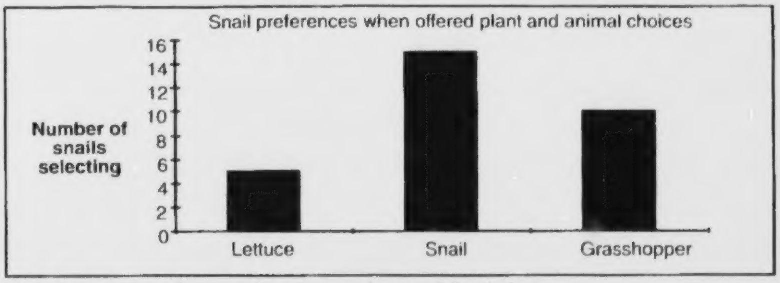
Answer to Test Question

Straw contains few nutrients and may not be very attractive to snails. The experimental animals expressed a strong preference for decaying lettuce. They may be attracted strongly to this garden plot and consume the crop when the decayed lettuce is exhausted. The lettuce-mulched plot may be predicted to have the greatest damage. However, if the snails cannot move between plots, the greatest damage will occur in the straw-mulched plot because snails do eat fresh vegetables.

TEACHING TIPS

- The recovery of animal material from the gut of field-collected snails does not seem to be the result of accidental ingestion. More than 20% of the specimens of one grassland species of herbivorous snail contained animal parts in their guts (Runham, 1975). These parts included both segmented worms and arthropods.
- *Lymnaea stagnalis*, the great pond snail, although primarily an herbivore, consumes dead animals. It has an ability to detect animal material at a distance, but not plant material (Runham, 1975).
- Snails may be omnivorous, but most are herbivorous.
- Although you could puree the materials for this exercise, the field snails will probably not encounter the food processed so completely. A "stepped-on colleague" is more likely to be the animal food encountered.
- *Helix pomatia* hatchlings devour unhatched eggs (Cain, 1983) and several other species of snails act as predators on occasion.
- Remember that if you want to do a Chi-Square Goodness-of-Fit, have expected values of at least 5 for each cell. Use this desired expectation to determine the number of trials and temperatures you will test. You will want to combine Lettuce 1 and Lettuce 2 for the statistical analysis. Since the snails have an equal opportunity of choosing one of four stations in the sample below, the expected value for lettuce should be half the number of snails observed.

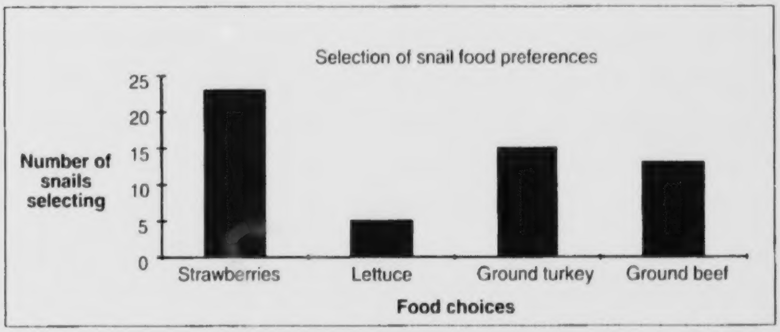
A Chi-Square Goodness-of-Fit analysis is appropriate for this design (see Variation 4).



Graph J. Snail preferences for plant and animal food choices.

TEST QUESTION

Graph K represents the results of an experiment testing snail food preference. Do these snails show a preference for any particular food? If so, which food do they prefer? How do these results compare with those you obtained?



Graph K. Snail preferences for 2 plant and 2 animal food choices.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Offer the snail two circles of paper with food choices to allow room between the foods. Make a third run with the best choices on other papers to determine final choices.
- See Variation 4 for additional modifications.

Deaf or Hard-of-Hearing

- See Variation 4.

VARIATION 9

The Effect of Mechanical Disturbance on Snail Activity

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS

Students will perturb snails gently with shell percussion and shaking to determine if these disturbances influence behavior.

ADDITIONAL MATERIAL NEEDED

You will need the following for each group of two students in a class of 24:

- 1 8-cm cotton swab
- 1 clock with second hand

HYPOTHESIS GENERATION

Question

Will snails respond to mechanical stimulation?

Interpretation

Reject the hypothesis if the same number of animals select the plant and animal foods or if more snails select the snail and grasshopper than select plant material. Accept the hypothesis if more snails select lettuce than select snail and grasshopper combined.

Answer to Test Question

Snails show a preference for strawberries and animal foods. Here, only half the snails chose animal foods.

TEACHING TIPS

- Some researchers (Kerkut & Walker, 1975) have observed snails withdraw in response to mechanical vibration, but others (Machin, 1975) working with hibernating snails have observed activation by percussion, shaking, or rotation.
- Snails can habituate to a stimulus (Kerkut & Walker, 1975), so do not stimulate the same snail in the same way more than 20 times.
- Turn off the lights in the classroom and increase the relative humidity in the vicinity of the snails for this activity. If you started with estivating or hibernating snails, they will be more likely to continue an initiated arousal if you provide conditions under which they would be active normally.
- Expect active snails to move 2 to 10 cm per minute (Jones, 1975).
- A team of four students can collect data quickly on this experiment if they work with a dozen snails. Three students could deliver the percussive stimulus, while the fourth student gently shakes three snails on the same plastic sheet.
- Try this with your unresponsive snails. If you still have trouble arousing them, lower their temperature to about 10°C and increase their relative humidity to 100% for four hours. This activated more than half the snails in one experiment with 48 animals (Machin, 1975).

Sample Hypothesis

Snails will exhibit defensive withdrawal behavior if they are disturbed mechanically.

Rationale

Turtles have a shell and often withdraw into it if disturbed. Disturbances, such as a tap, may be the initial encounter with a hungry mouse, bird, frog, toad, lizard, or salamander. Hiding in the shell would be a better strategy than outrunning one of these predators.

Sample Experimental Procedure

1. Place a snail on a glass plate or plastic sheet and mist the surface lightly with spring water.
2. Provide no further stimulation to 1 snail.
3. Stimulate each of the remaining 2 snails continuously for 2 minutes using a different stimulation technique for each snail as follows:
   - A. For percussive stimulation, rest a cotton swab midway between 2 fingers with one tip of the swab just resting on the back of the snail's shell. *Gently*, flick the free end downward about 10 times per minute. See Figure 19.
   - B. For shaking stimulation, lift the plastic sheet off the lab bench and move it *gently* left to right and back through a displacement of about 15 cm, then pause. Repeat this process at the rate of 10 complete displacements per minute.
4. As soon as you have finished stimulating your snail, place a piece of lettuce or the preferred food 10 cm from its anterior end. Record the rate of movement toward the food. Continue your observation for 5 minutes, moving the lettuce just beyond the reach of the snail if necessary.

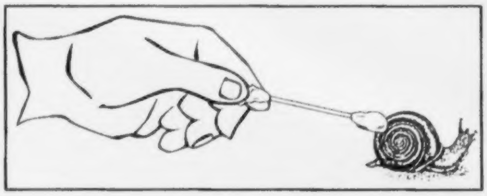


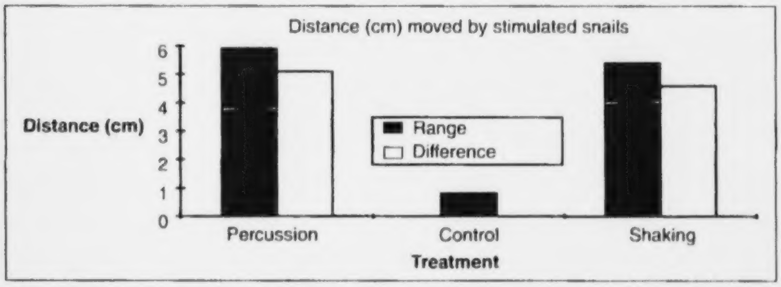
Figure 19. Procedure for using a cotton swab as a percussive stimulation.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 18. Distance moved and behavior exhibited by mechanically stimulated snails.

Trial	Distance moved with treatment		
	None (cm)	Percussion (cm)	Shaking (cm)
1	0 No activity	10.0 Withdrew, then emerged and moved quickly	3.4 Did not withdraw
2	0 No activity	2.3 Withdrew, then emerged and moved, but stopped after a short distance	5.2 Did not withdraw
3	2.3 Moved toward lettuce	5.5 Withdrew, then emerged and moved slowly	7.5 Did not withdraw
Mean	0.8	5.9	5.4



Graph L. Effect of mechanical stimulation on snails.

TEACHER'S NOTES

Interpretation

Reject the hypothesis if some snails withdrew and some did not, and discuss what was observed. The snails responded differently to the two kinds of mechanical stimulation. Accept the hypothesis if stimulated snails withdrew into their shells.

TEST QUESTION

What natural changes in the environment might result in mechanical stimulation? What snail responses to these stimuli might be adaptive in the field?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have students create mechanical vibration and other disturbances for the snails. The braille watch can be used as a timer.
- Tape braille letters to the shell of each snail as a label when more than one snail is being used. Drops of glue also may be used as a marking device on the shells.

VARIATION 10

The Effect of Surface Texture on Snail Movement

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.

SYNOPSIS

Students will determine whether snails move at the same rate over smooth and coarse surfaces.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- waterproof sandpaper of various grit sizes
- 1 tape measure or ruler and string
- 1 clock or watch with second hand

HYPOTHESIS GENERATION

Question

How does the texture of a surface affect snail movement?

Sample Hypothesis

Snails will move more slowly over a rough surface than over a smooth surface.

Rationale

A smooth surface should provide better contact with the mucus and cilia that are involved with the snail muscles that create movement.

Sample Experimental Procedure

1. Mist each selected sandpaper with spring water to create a wet surface without standing water. You may blot the surface with paper towels to remove excess water.
2. Place a snail on the sandpaper 5 cm from a preferred food.
3. Record the time required for the snail to reach the food.
4. Calculate the rate of movement in centimeters per minute.

Answer to Test Question

Mechanical stimulation could simulate the movement of vegetation at nightfall or from an arriving storm. It might be the tap of a rain drop. If these changes are initiated by the stimulation, it would be advantageous for the snail to increase its activity of foraging for food with little risk of dehydration. However, if the mechanical stimulation is the result of a predator testing the shell for contents, it would be advantageous for the snail to withdraw. It is unlikely that it can outrun a predator.

TEACHING TIPS

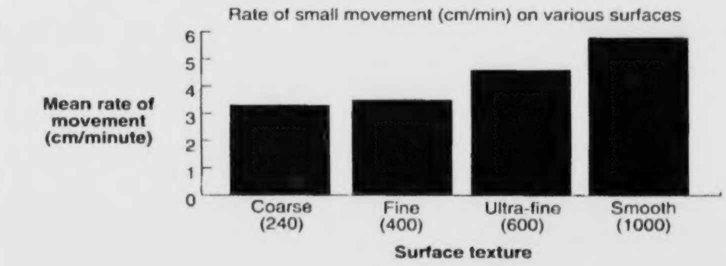
- Waterproof sandpaper is not made in extremely coarse grits, but other sandpaper tends to come apart when wet.
- You are more likely to find waterproof sandpaper in a variety of grits at an automobile parts supply store than at a hardware store.
- If the snail does not travel in a straight line, you may measure how far it went by laying a string along its route and then measuring the string.
- The main adhesion between a land snail and the surface that it moves over is the mucus distributed over the foot surface by cilia. The mucus appears to be necessary for movement (Jones, 1975).
- Within a species, small individuals move faster than large individuals (Hyman, 1967). *Helix aspersa* moves about 7.5 cm/minute, and *Helix pomatia* moves from 6.25 to 7.8 cm/minute.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 19. Rate of movement (cm/minute) for snails on various surfaces.

Trial	Smooth (glass) cm/minute	Ultra-fine grain (600) cm/minute	Fine grain (400) cm/minute	Coarse grain (240) cm/minute
1	5.2	5.0	4.5	4.2
2	5.0	5.1	4.0	2.0
3	7.1	3.8	2.0	3.8
Mean	4.4	4.7	3.6	3.3



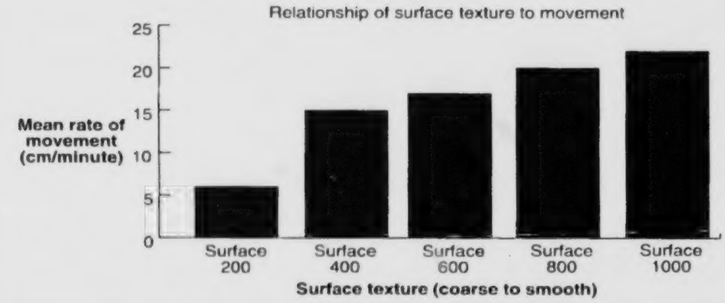
Graph M. Influence of surface texture on the rate of snail movement.

Interpretation

Reject the hypothesis if the average rate of movement on any textured surface is greater than that on the glass surface. Accept the hypothesis if the slope of the line is negative.

TEST QUESTION

Study the graph of snail movement in Graph N. Compare the rate of movement of these snails over various surfaces. Is the performance of these snails comparable to that of your experimental animals?



Graph N. Influence of surface texture on the rate of snail movement.

Answer to Test Question

The smoother the surface, the faster these snails are able to move. Our snails showed the same pattern of change in rate, but they moved much more slowly.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide braille timing devices with a second hand if available. The class timer used in science labs is usually sturdy enough to be used manually by the student who is blind.
- Provide braille tape measures and rulers.
- Use braille graph paper for a line graph. The points are marked on the paper and are connected with a French curve.

A Study of Snail Behavior

Directions for Students

INTRODUCTION

Have you ever observed animals in action? Bees moving towards a flower, ants toward food, a snail towards a leaf, or a roach darting for cover when a light is turned on? Why are these animals exhibiting these behaviors? How do they "know" where to go? Is it instinct? Have they learned these behaviors?

Most of us are aware of the senses of touch, taste, smell, seeing, and hearing. Which sense do you use the most? Which of your senses is the most reliable? Do other animals also have these senses? In this activity your teacher will provide you with a snail to observe. Spend 5 to 10 minutes watching the snail. As you watch your snail, how could you test whether a snail is able to sense its environment?

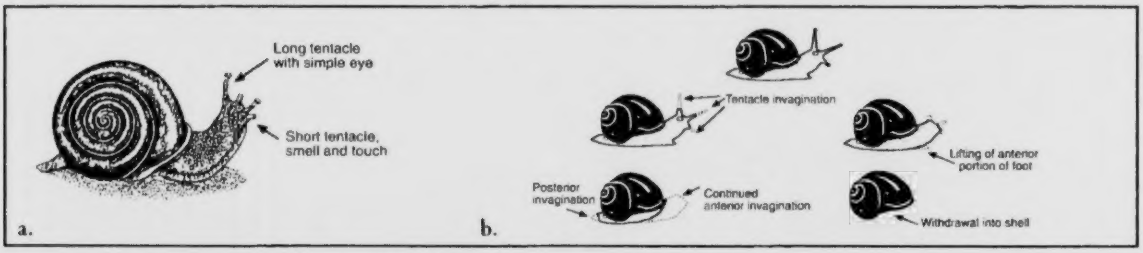

Long tentacle with simple eye
Short tentacle, smell and touch
a.
b.
Tentacle invagination
Lifting of anterior portion of foot
Posterior invagination
Continued anterior invagination
Withdrawal into shell

Figure 1. a. Snail with tentacles' functions identified. b. Normal retraction sequence in snails.

OBJECTIVES

At the end of this lab, you should be able to:

- Determine how snails respond to chemical stimuli, including chemicals found in their environment.
- Discuss why snails may respond differently to various chemical stimuli in their environment in terms of evolution.

MATERIALS NEEDED

For each team of two students, you will need the following:

- 1 1-L plastic or glass jar with lid or 1 3 x 10 x 10-cm plastic sandwich container with snap-on lid
- 1 8 x 8-cm piece of 2-cm thick Styrofoam™
- 1 compass with pencil
- 2 25-cm filter paper pieces, paper towels, or coffee filters
- 1 mister/sprayer
- 1 large land snail (*Helix)*
- 1 20 x 30-cm plastic sheet protector, report cover, or glass plate
- 1 25-cm diameter glass pie plate or clear plastic dish
- 1 permanent marker
- 1 metric ruler
- 1 pair of scissors
- 10 mL spring water
- 3 25-mL vials
- 1 forceps
- 1 clock or watch with second hand
- 10 mL 10% spinach extract
- 10 mL 10% glucose solution
- 1 lab journal

SAFETY PROCEDURES

- Use only substances that can be poured safely down the sink to make disposal easier.
- Do not use chemicals that are toxic or dangerous to humans.
- Never use pure caffeine or nicotine. Avoid pesticides and insecticides.
- Do not release snails into gardens or greenhouses. Both *Helix aspersa* and *Helix pomatia* are European natives that are serious agricultural pests.
- Do not permit snails to crawl on lab benches.
- Wear safety goggles and aprons when working with chemicals.
- Students should wash their hands before and after working with the snails.
- Keep hands and fingers away from the compass point when punching holes in lids.
- Handle snails with care. Never pull or lift the animal directly from a surface. Rather, gently slide the animal from a surface.
- Always maintain a moist environment. Clean container at least once a week. Do not use distilled water or tapwater directly on the snails.

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STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of snails and chemical sensitivity. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. What makes this question an interesting or important topic for investigation?
3. Why is controlling variables important?
4. What is the variable in your experiment?
5. How will you control other potential variables?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. Combine your results with those of all your classmates. Construct a bar graph showing the number of snails responding positively and negatively to each chemical tested.
2. Do your data support or refute your hypothesis? Explain.
3. In one set of student experiments, 100% of the snails showed a positive response to spinach; while in another set of student experiments, 75% of the animals showed a negative response. What explanations might account for the difference?
4. Would knowing that the sample size in one experiment was 30 snails, while in the other experiment it was 4 snails, change your confidence in the results? Explain.
5. How could you test whether the aroma of the material influences the movement of a snail toward it?

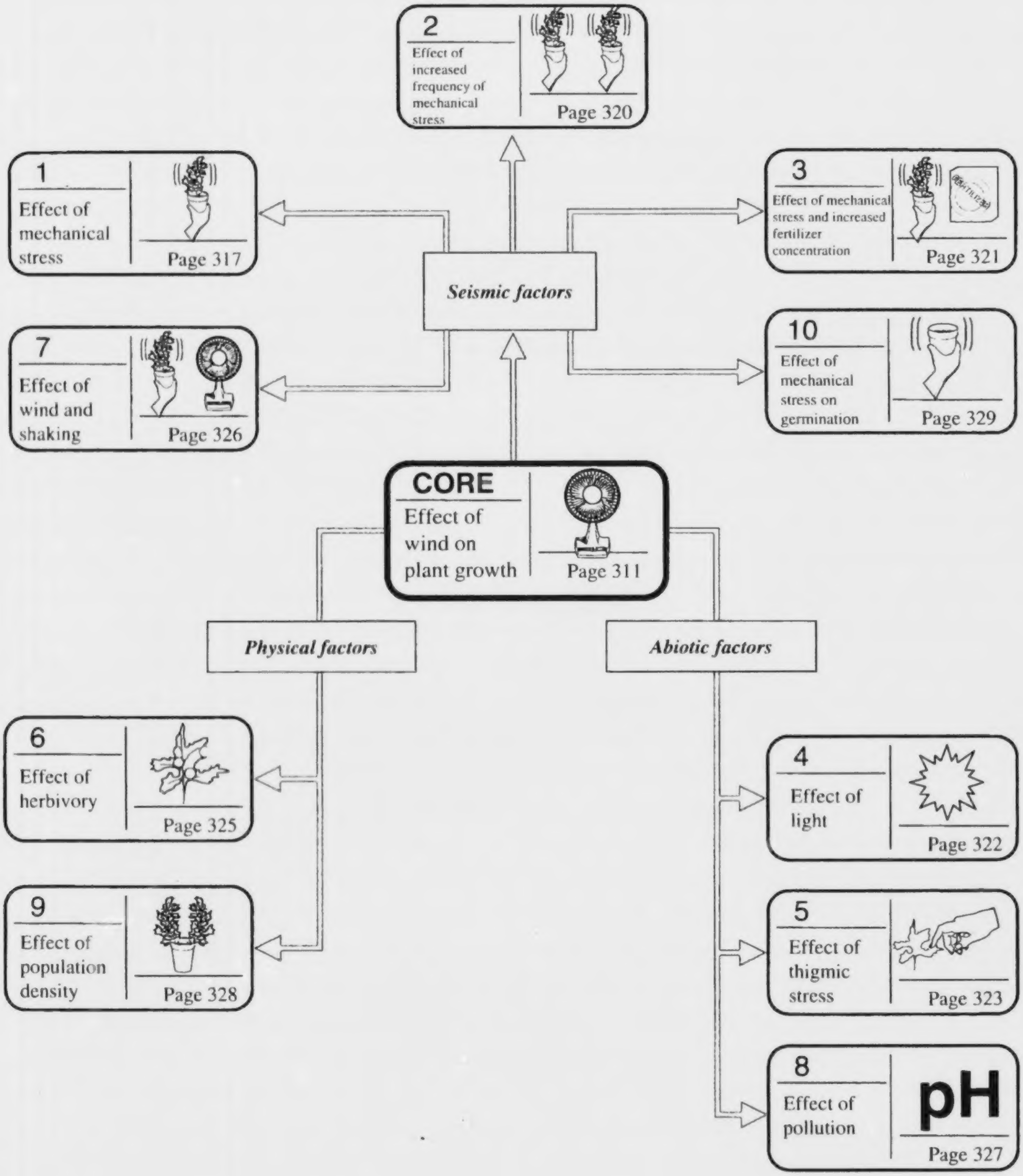
DESIGN OF VARIATIONS OF CORE EXPERIMENT

After collecting and analyzing the data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for experiments you could do next. Think of questions that occurred to you as you conducted the Core Experiment on snails and environmental factors. What quantifiable experiments could be done based on the observations you made? Questions other students have studied include the following:

- How will changing the solution's concentration affect the snail's response?
- What type of geotactic response do snails exhibit?
- What form of phototactic response do snails exhibit?
- Are snails able to detect different colors?
- Do snails have a temperature preference?
- Do snails avoid food that is toxic?
- Do snails prefer fresh or decayed food?
- Will snails prefer plant material over animal material?
- Will snails respond to mechanical stimulation?
- How does the texture of a surface affect snail movement?

Stressed Out Plants

At-A-Glance Map of the Core Experiment and Its Variations


2
Effect of increased frequency of mechanical stress
Page 320
1
Effect of mechanical stress
Page 317
3
Effect of mechanical stress and increased fertilizer concentration
Page 321
Seismic factors
7
Effect of wind and shaking
Page 326
10
Effect of mechanical stress on germination
Page 329
CORE
Effect of wind on plant growth
Page 311
Physical factors
Abiotic factors
6
Effect of herbivory
Page 325
4
Effect of light
Page 322
9
Effect of population density
Page 328
5
Effect of thigmic stress
Page 323
8
Effect of pollution
pH
Page 327

NOTES

Stressed Out Plants

SYNOPSIS FOR CORE EXPERIMENT

Students will determine if wind of various intensities affects plant growth.

APPROPRIATE BIOLOGY LEVEL

Introductory or advanced

TEACHER PARTNERS
Feagin Fuqua and Dean Medley
Eastern Guilford High School
415 Peeden Drive
Gibsonville, NC 27249-9218

SCIENTIST PARTNER
Lee Williams
Alamance Community College
PO Box 8000
Graham, NC 27253

Directions For Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 317. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Describe the impact of wind stress on plants in different habitats.
- Discuss the effect of wind stress on plant growth.

MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 4 6-cm round pots
- 4 labels
- 100 g potting soil
- 4 tomato plants, 5 cm in height
- 2 spoons
- 1 metric ruler
- 1 50-mL graduated cylinder
- 1 automatic timer
- 1 journal or sketch pad
- 1 marking pen
- 1 spray water bottle
- 1 fan with 3 different speed levels

You will need the following for a class of 24 students:

- 8 sheets of newspaper
- 1 kg (2.2 lb) soluble plant fertilizer
- 1 fluorescent plant light, greenhouse, or sunny window
- 1 wall clock with second hand or 1 timer

SAFETY PROCEDURES

Follow the instructions carefully when preparing the fertilizer solution.

Wash hands after handling the fertilizer.

DIRECTIONS FOR SETTING UP THE LAB

Fertilizer Solution

Mix 3.8 L (1 gallon) of fertilizer solution according to instructions.

TEACHER BACKGROUND

Content Information

Water, nutrients, light, carbon dioxide, and oxygen are all necessary for optimal plant growth. A shortage of any one of these stresses plants, and may reduce their growth or ability to reproduce. Though some types and levels of stress may benefit plants, the effects of stress are usually negative. Every year, stress to crop plants accounts for billions of dollars in agricultural loss. Sustained periods of severe environmental stress, such as drought, can destroy crops and cause famine.

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Plant the tomato plants. Measure the initial plant height, mass, and number of leaves. Subject the plants to stress for varied periods of time.

Days 2 to 10 (15 minutes)

- Measure the height, mass, and number of leaves. Subject each plant to the appropriate amount of stress.

PREPARATION TIME REQUIRED

6 to 8 weeks ahead

- If growing rather than purchasing plants, start seeds.

1 hour

- Prepare and gather the materials needed for the Core Experiment.

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TEACHER'S NOTES

Other sources of stress to plants include pests, pollution, disease and extreme temperatures. One type of stress that often is overlooked is mechanical stress. Plants respond to various forms of touching, bending, and shaking caused by such things as rain, wind, or insects. Touching a plant stimulates reactions known as thigmic responses. Well known examples of this are the closing of the Venus flytrap or the folding of the Sensitive Plant (*Mimosa*) leaves when touched. Shaking a plant without touching it produces seismic stress. This type of stress can be induced by wind or earthquakes.

It is easy to see the effects of seismic stress on plants that grow in windy places, such as beaches. Trees growing there are often short and deformed. Growth on the windward side of the plant is stunted while growth on the leeward side is more lush. See Figure 1. Other environmental stresses, such as salt spray, add to the effects of the wind on coastal plants. Also, the sandy soils do not retain water or nutrients very well, so coastal plants are vulnerable to droughts.

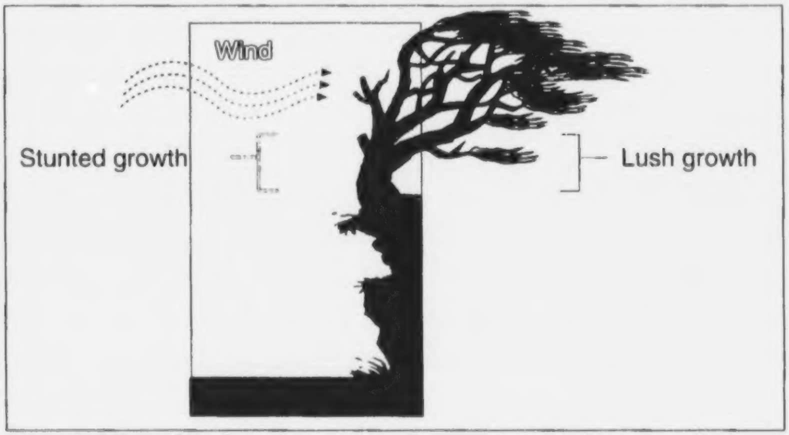


Figure 1. Effects of beach wind on tree growth.

Another example of the effect of wind on plant growth can be seen in forests that have been cleared by logging. In some areas, loggers remove taller trees, leaving behind smaller understory trees. The taller trees that were exposed to the wind as they grew responded by developing a strong cellular structure in their trunks; they slowly grew straight, tall, and wind resistant. On the other hand, the smaller understory trees were sheltered from the wind by the taller trees and did not develop the same type of strong cellular structure in their trunks. After the large trees are harvested, the smaller ones are exposed to wind for the first time. It easily bends their slender, relatively weak trunks.

Research done in the 1970s showed how different forms of mechanical stress affect plants (Decker, 1990). The effects were generally found to be negative, such as decreased growth rate, reduced leaf area, and lower biomass. In 1990, scientists determined more specifically how plants actually respond to stress (Beardsley, 1990). When plants are stressed, genes are activated to produce certain proteins. One of these proteins is calmodulin which is involved in calcium metabolism. It has been speculated that calcium ions are involved in the response of plants to stress.

Based on these findings, scientists devised a more precise method of observing the responses of plants to stress (Zimmer, 1992). The researchers inserted a gene from a jellyfish into the plant cell's genome. The jellyfish gene produces a protein called aequorin that glows when it reacts with calcium ions. This technique can be used to detect stress-induced increases in calcium ions. Thus, when disturbed, these genetically engineered plants emit a burst of light.

Could glowing plants one day line the runways of airports? Could Christmas trees be grown that light up when fanned? Could farmers observe how stressed their crops are by checking the glow from the fields at night? The likelihood of developing any of these applications is remote because the light emitted by these genetically engineered plants is so dim that it cannot be detected except with specialized instruments. However, glowing plants have proved valuable as research tools, and scientists are working on ways to increase the amount of light they produce.

Research in plant responses to mechanical stress also has more practical applications in commercial agriculture. For example, tomato plants tend to grow long, weak stems when grown hydroponically; chemical growth retardants alleviate this problem but often reduce yield as well. As an alternative, commercial growers are exploring the use of wind stress applied with a sweeping motion by a leaf blower to reduce plant "legginess" without decreasing fruit production. Home gardeners may be able to use similar methods to keep plants such as chrysanthemums from becoming too tall and falling over (Brune, 1991).

Pedagogical Information

The following is a chart of a concept related to this lab and a student misconception of this concept.

Correct Concept	Misconception
• Plants can be affected by a variety of environmental stresses, including wind.	• Plants do not demonstrate physical symptoms of stress.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Obtain videos that illustrate stress on living things, such as earthquakes, hurricanes, or wind storms. Show pictures of plants affected by mechanical stress to illustrate graphically new terms and concepts. See Figures 3 and 4. Have students locate pictures from magazines, posters, or newspapers.

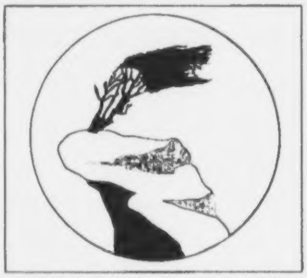


Figure 3. Wind flagging of trees on mountain tops.

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

Ask the following questions as a lead into hypothesis generation:

- What do all the plants in the pictures have in common?
- Do you think plants on the coast grow differently from those in inland areas?
- Do plants grow differently in greenhouses than outdoors?
- Are plants near airport runways affected by the wind, sound, and pollution from planes? If so, what evidence would you look for to demonstrate this?
- Are plants near railroad tracks affected by the movement of trains on the tracks? If so, how could you demonstrate this?
- Are plants on the edge of a highway affected by the heat, exhaust, and vibrations of cars and trucks? If so, how could you demonstrate that they were?

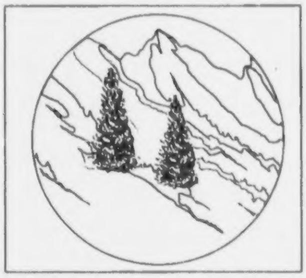


Figure 4. Trees not affected by wind flagging.

TEACHING TIPS

- This activity can be integrated easily into the following subjects: Physical Science, Environmental Science/Ecology, Computer Science, or Statistics.
- Contact a local nursery for the tomato plants. Some are willing to donate them for education. Be sure to acknowledge their generosity with a letter to the principal and local newspaper.
- It is easiest to conduct this lab in the spring when tomato plants are readily available. If tomato plants are not available, plants can be grown from seeds. The germination time is about two weeks. A height of 5 cm is reached within six weeks of planting.
- Sunflowers or "fast plants" (*Brassica rapa*) can be substituted for tomato plants.
- Milk cartons from the cafeteria can be used for pots. Punch small holes in the bottom for drainage.
- Plants exposed to wind may show the effects of water loss due to wind stress. This could lead to additional investigations.
- Inexpensive growth chambers may be made from plastic storage containers available in a variety of sizes from discount stores. Locate a drill bit the size of the hole needed to insert the light fixture. Plant lights, such as Gro-lux,® will work well. See Figure 2.

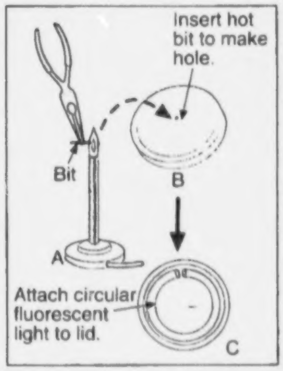

Insert hot bit to make hole.
Bit
B
A
Attach circular fluorescent light to lid.
C

Figure 2. Steps for constructing an inexpensive growth chamber from a plastic storage container. A. Hold the bit with pliers and heat over a Bunsen burner. Wear pot holders on your hands to protect from the heat. B. Hold the bit with the pliers and push it through the lid. C. Insert the fixture. A circular fluorescent growth light works well.

TEACHER'S NOTES

Sample Hypotheses

- If tomato plants are exposed to wind, frequently they will develop shorter, sturdier stems.
- If tomato plants are exposed to wind, they will produce fewer leaves than plants that are not exposed to wind. The number of leaves produced will decrease as the time exposed to wind increases.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 315.

Question

What effect do varying intensities of daily winds have upon plants?

Hypothesis

If plants are subjected to varying intensities of wind daily, the growth of plants exposed to the most intense winds will be affected negatively.

Rationale

In windy locations, small and distorted plants are observed often.

Procedure

1. Cover the desk with newspaper.
2. Label 4 pots as follows: A—Control, no treatment; B—Expose to low setting; C—Expose to medium setting; and D—Expose to high setting.
3. Plant the tomatoes by carefully placing 1 plant in each pot and covering its roots gently with 15 g of soil. Compact the soil slightly. Then add 10 g of soil.
4. Moisten the soil with water from a spray bottle.
5. Measure the height of the stem and count the number of leaves for each plant.
6. Place the tomato plants in a tray of fertilizer solution under a light source. Regulate 12-hour light and 12-hour dark periods with an automatic timer.
7. Subject plants to wind stress with an oscillating fan or fixed fan at a distance of 60 cm (2 feet) for 40 minutes or up to 3 hours per day. Use masking tape to insure proper placement of the fan or other item daily. See Figure 5.

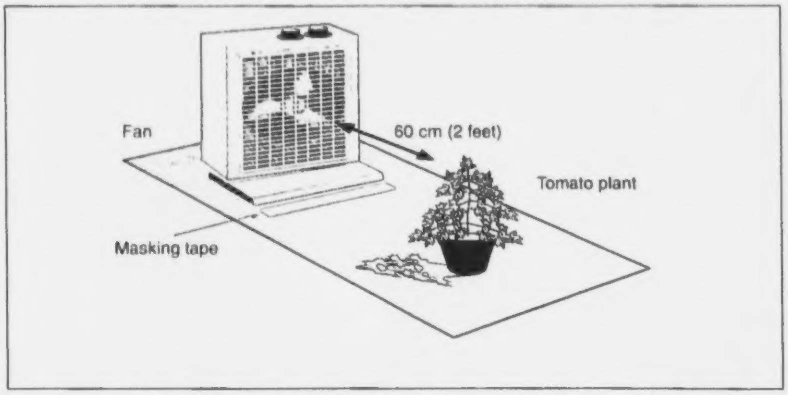


Figure 5. Fan placement for wind stress experiment.

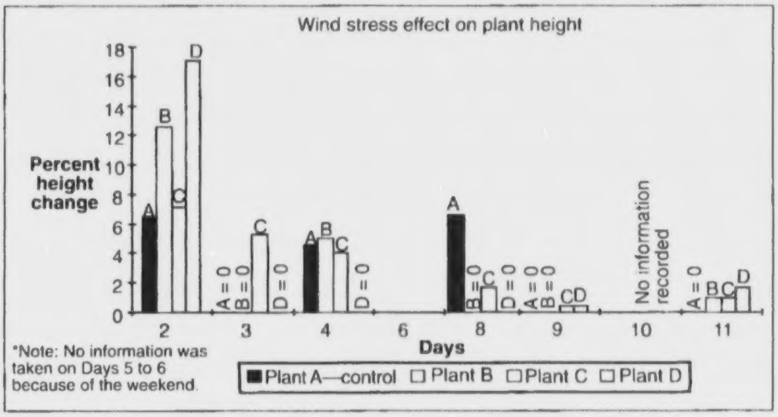
8. Place the plant under a plant light with timer or in a sunny window. All of the environmental parameters with the exception of wind stress, should be the same for all plants, including the amount of water added to each pot.

9. Repeat the wind stress for 11 days. Instructors may want to use a timer to turn on fans to insure a consistent exposure to wind.

10. Collect data regarding the height of the plants, the number of leaves, and size of sample leaves obtained from the sides toward and away from the fan. Students may measure the diameter of the stem at specified distances from the soil surface. In addition, students may devise a way to measure the strength of stems, such as adding weights to a stem placed horizontally in order to determine its strength.

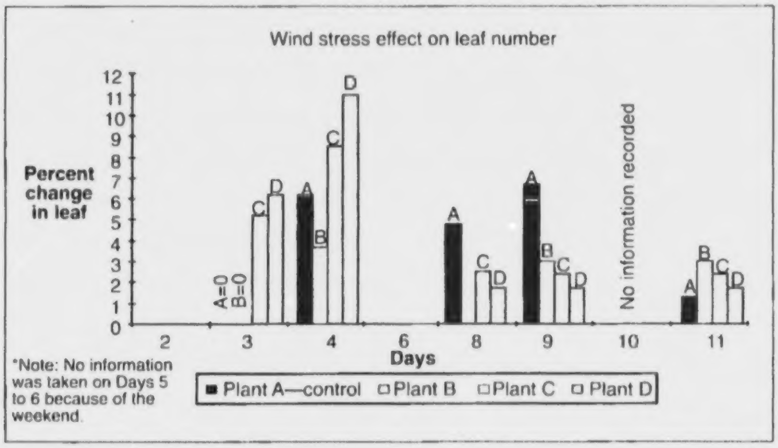
DATA ANALYSIS AND INTERPRETATION

Sample Data


Wind stress effect on plant height

*Note: No information was taken on Days 5 to 6 because of the weekend.

Graph A. Wind stress and plant height.


Wind stress effect on leaf number

*Note: No information was taken on Days 5 to 6 because of the weekend.

Graph B. Wind stress and leaf number.

TEST QUESTION

Four students designed an experiment to test wind stress on pea plants. They selected 4 plants with approximately the same height and mass. One of the plants (A) was selected as a control, and the other 3 plants (B, C, and D) were subjected to wind from a hair dryer for 30 minutes twice a day for 10 days. The plant heights of the 4 plants were determined on the 11th day. Analyze their results in Graph C and choose the correct answer.

Interpretation

Wind stress appears to increase height initially. When plants are subjected to long periods of wind, stress is not a factor in increased plant height.

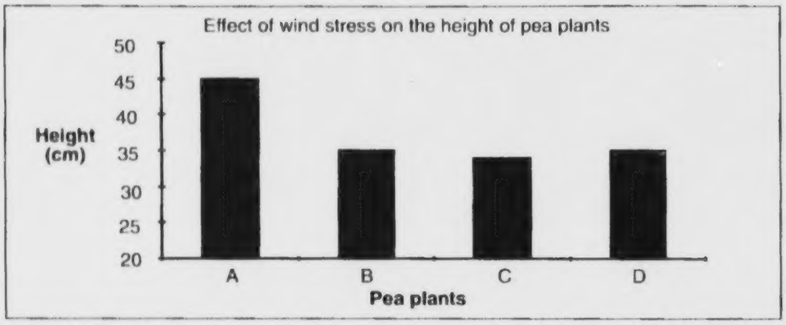
Interpretation

There seems to be an optimum time for increased leaf number between the third and fourth days of exposure.

Answer to Test Question

D.

TEACHER'S NOTES



Graph C. Pea plants subjected to wind stress.

The wind-stressed pea plants showed:
A. a greater increase in mass than the control plant.
B. no increase in mass over 10 days.
C. a greater increase in height than the control plant.
D. a smaller increase in height than the control plant.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After students have collected and analyzed the data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted their first experiments. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- What effects, if any, did wind have on your plants?
- Based on your results, what effect on the growth of plants would you expect to find in windy habitats such as the seashore, on mountains, or on the prairies?
- In some species, plants exposed to intense winds develop stronger stems than plants not exposed to wind. How might you measure whether your plants exposed to wind had stronger stems? *Note to teacher: Students could measure the diameter of the plants' stems at the same height above ground. Or, students could measure the weight needed to bend the stem.*
- Are some plants more sensitive to wind stress than others? How would you determine this? For instance, would you expect plants with broad leaves or narrow leaves to be more sensitive to wind stress?
- Does exposure to air pollution stress plants?
- How does exposure to temperature fluctuations stress plants?
- What effect would increased precipitation have on these same plants in the windy habitats? What findings of your investigation helped you answer this question? Would you expect plants exposed to the highest intensity wind to grow as well as your control plants if the plants exposed to wind were watered more?
- Wind is an environmental stressor. What other natural environmental stressors might affect the growth of plants? What human-caused environmental stressors might affect the growth of plants?
- How can plants be used to determine the environmental conditions of the habitats where they are found?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p.15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

- Provide, where possible, each student with their own braille rulers and timers. Students who are accustomed to helping at home with house work and yard work should be able to perform every detail of the Core Experiment—working alone, or as part of a team. Previous math or science courses should have given them experience in making braille graph paper on their brailler and using this to make bar graphs.
- Stress the different texture of leaves, where possible. Often, this is noticed by the students who are visually impaired before others in the class are aware of it.
- Provide a source of labels to use with their brailler so they can affix suitable labels on their plants.
- The blind student is likely to have a record player for use with Talking Books but it should not be used in the experiment. Shaking plants by hand to the beat of a metronome should be as satisfactory.

Deaf or Hard-of-Hearing

- Provide deaf students with a complete copy of the instructions for the experiment. They should be assigned to a congenial group of students who will converse by note pad, when necessary. No special modifications are needed. See "Allelopathy" for other suggestions.

Gifted

- Design a wind block to study the beneficial effects on plants.
- Determine a threshold for plant stress.
- Introduce computer-generated graphs and graphic analysis.

Manually Impaired

- Assist students who are manually impaired with planting seeds, caring for plants, and measuring plants. The use of larger seeds may be better for some. Good lab partners will be able to include them in the investigation.

Mobility Impaired

- See "Alleopathy" for suggestions.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. How might stunted growth be beneficial to a plant that is subjected to mechanical stress?
2. Two tomato plants were shaken daily with the same intensity as they grew. Plant A received 1 minute of shaking, while plant B received 3 minutes of shaking. Which plant would you expect to have grown taller after 2 weeks? Which would you expect to have a thicker stem? Why?

Answers to Additional Test Questions

1. Stunted growth may protect plants from severe damage caused by the stress. For example, trees growing in windy areas are less likely to be blown over if they are short and stout.
2. The increased mechanical stress will cause plant B to be shorter and thicker-stemmed to better support it against stress.

Answers to Questions and Analysis on Student Page

1. Musical vibrations are a source of seismic stress, just like traffic or earthquakes.
2. These stresses probably will affect plant growth negatively.
3. There is shorter, scrubbier growth due to wind and salt stress, possibly drought stress in poor sandy soil.
4. They are shorter and stouter due to wind stress; there may be uneven growth with stunting on the side that faces prevailing wind.
5. It will harm the small trees that are not adapted to withstand mechanical stress from wind and rain.
6. It will remove wind stress, so plants will grow more quickly in height. It also could permit even growth on all sides of the stem.

REFERENCES AND SUGGESTED READINGS

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POSSIBLE SOURCES OF MENTORS

Agricultural extension agent
Local college or university professor
Farmer
Plant nursery owner

VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

***Note to Teachers:** Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.*

VARIATION 1

The Effect of Mechanical Stress on Plant Growth

SYNOPSIS

Students will shake tomato plants to determine the effect of mechanical stress on the plants' height, mass, and leaf size. They will compare these parameters among plants subjected to different duration of mechanical stress with a control.

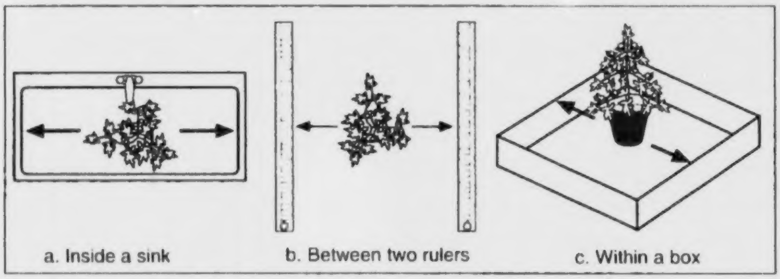

a. Inside a sink b. Between two rulers c. Within a box

Figure 6. Suggested techniques to establish consistent motion and speed.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24:

- 1 plant-shaking apparatus
- 3 balances
- 1 roll of photograph film *(optional)*
- 1 camera *(optional)*
- 1 metronome or aerobics audiotape *(optional)*
- 4 L water
- 1 bucket
- 3 paper towels

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Set up rotary shaker. Alternatively, students may shake plants by hand. See Teaching Tips for suggestions or a plant-shaking device may be constructed as shown in Figure 7.

(*Optional*) Figure 7 shows a possible design of a plant-shaking device using a record player. This setup will work best with a direct drive turntable. The speed and torque of most record players are very low and may not be suitable for some applications.

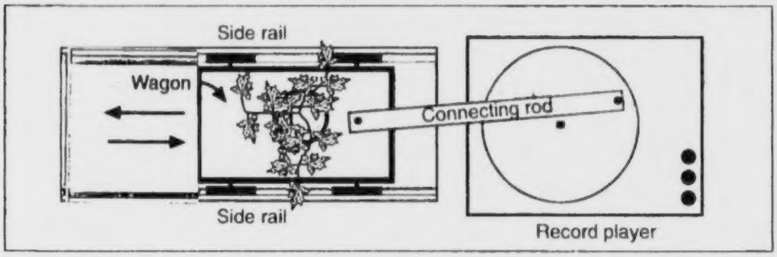


Figure 7. Plant-shaking device.

PREPARATION TIME REQUIRED

1 hour

- Locate a plant-shaking machine

5 hours: (optional)

- Construct the plant shaking device described in the Directions for Setting Up the Experiment.

TEACHING TIPS

- Plants should be kept in an area where they will not be disturbed by students or the wind. When handling the plants, students should avoid extreme shaking or touching that may damage the plants.
- Borrow a rotary shaker from a local university or medical laboratory. Most biochemistry/microbiology labs have several.
- A plant-shaking device can be constructed from a record player turntable (see Figure 7), or use a spice rack turntable to simulate the motion of the phonograph turntable.
- Students can shake the plants by hand, but it must be done with a consistent motion and speed. A shoe box, sink, or two rulers can be used to establish the boundaries. Figure 6 illustrates several ways to do this.
- Music can provide a steady rhythm. A tape designed for an aerobics class with the number of beats per minute stated is useful for establishing a constant rhythm.

BEST COPY AVAILABLE

TEACHER'S NOTES

1. Remove the needle from the phonograph arm. If possible, remove the entire arm.
2. Lift the platter from the turntable and drill a 0.5-cm hole into it about halfway between the center and the edge. Holes also can be drilled at different distances from the middle to alter the wagon's length of travel.
3. Use a piece of wood approximately 2.5 x 1 x 55-cm for the connecting rod. Drill a 0.5-cm hole into each end.
4. Obtain or construct a small wagon (9 x 12.5 x 30-cm) for the plant holder.
5. Construct a track to keep the wagon moving in a straight line using a piece of plywood (26 x 65-cm) and side rails (2.5 x 1 x 65-cm).
6. Attach the connecting rod to the platter with a screw, nut, and spacers to keep the rod above the center shaft of the turntable.
7. Connect the other end of the rod to the wagon using a nail. Connect both ends of the rod loosely to allow proper movement.
8. Adaptations of the above procedure are shown in Figures 8 and 9.

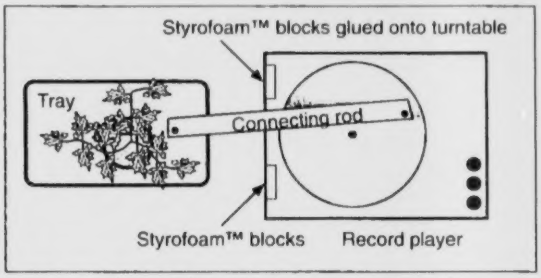
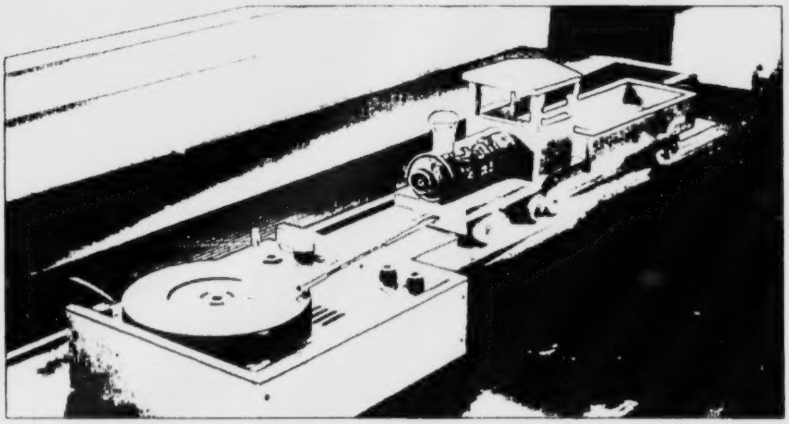


Figure 8. Plant-shaking device modification. The procedure is the same with the exception of the side rails. The motion of the tray is controlled by Styrofoam™ pieces affixed to the body of the record player.



Mark Temons

Figure 9. Plant-shaking device designed as a locomotive.

HYPOTHESIS GENERATION

Question

What effect does the mechanical stress of shaking have upon plant growth?

Sample Hypothesis

Tomato plants that are shaken will show less growth in height than plants that are not shaken. The height growth rate will decrease as the length of shaking time increases.

Rationale

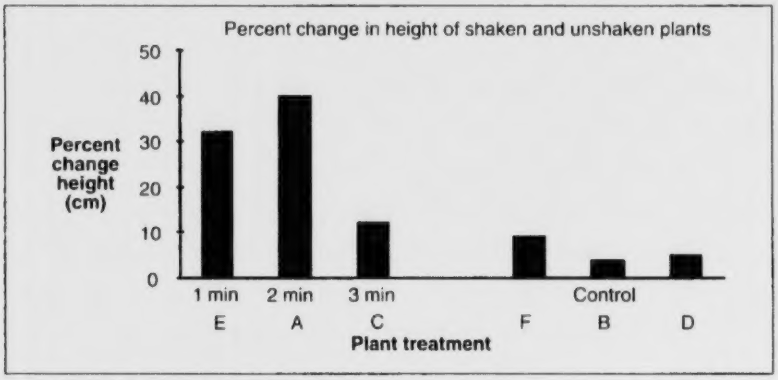
Plants will be stressed by shaking and their growth will be inhibited.

Sample Experimental Procedure

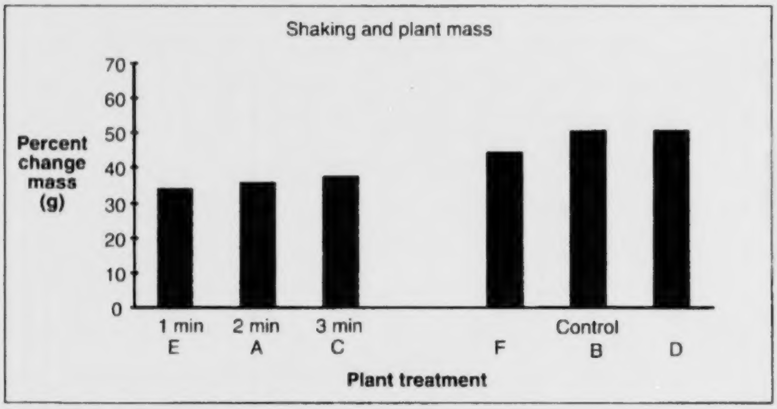
1. Measure the mass of the pot, soil, and plant; stem height; and number of leaves of each plant.
2. Sketch each tomato plant showing the leaves and stems. If possible, photograph them.
3. Shake each plant for the appropriate length of time (0, 1, 2, or 3 minutes) using a shaker device. If a plant shaker is not available, shake the plants by hand. Use a metronome or aerobics audiotape to regulate "shakes" per minute. Repeat each day for 2 weeks.
4. Remove the plants carefully from the pots at the end of 2 weeks. Free the roots from the soil by swirling them in a bucket of water. Dry the roots gently with paper towel. Take wet and dry weights of leaves, stems, and roots.

DATA ANALYSIS AND INTERPRETATION

Sample Data 1



Graph D. Shaking and plant height.



Graph E. Shaking and plant mass.

Interpretation

Shaking plants decreases plant mass, as expected, but plant height shows an increase with shaking.

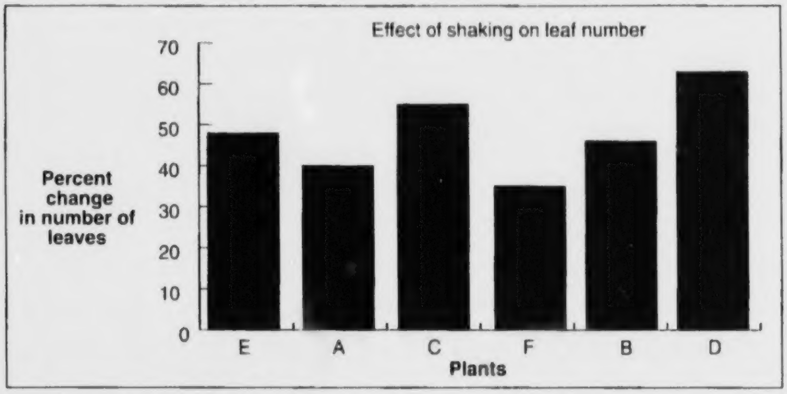
Interpretation

This shows that shaking does not significantly affect the number of leaves.

Answers to Test Questions

1. Answers may include wind, insect damage, cutting, rubbing, shaking, or rain. Each of these most likely would cause decreased height, mass, and leaf production.
2. Thigmic stress occurs when a plant is touched. Examples could include walking across a lawn and children grabbing plant leaves.
3. Seismic stress occurs when a plant is shaken without being directly touched, such as by wind or an earthquake.

Sample Data 2



Graph F. Shaking and leaf number.

TEST QUESTIONS

1. Identify and describe the effects of four sources of mechanical stress to plants.
2. What is thigmic stress? Give an example.
3. What is seismic stress, and how does it differ from thigmic stress?

VARIATION 2

The Effect of Multiple Seismic Events on Plant Growth

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will subject plants to shaking two to three times daily and determine the effects on growth relative to the control plants.

HYPOTHESIS GENERATION

Question

What effect will increasing periods of seismic stress have upon plant growth?

Sample Hypothesis

Plants subjected to mechanical stress will show a lower rate of growth in both height and leaf number when compared to a control. Increasing the frequency of periods of seismic stress will have a corresponding negative effect.

Rationale

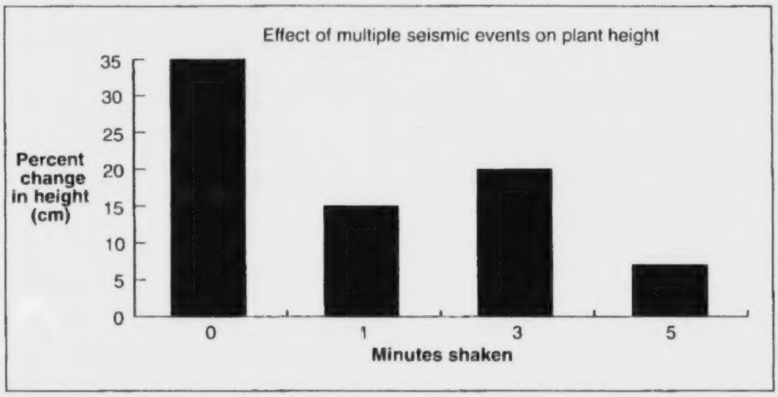
Increasing periods of stress should reduce plant growth.

Sample Experimental Procedure

Same as for Variation 1, with additional periods of stress. Suggested periods of stress are as follows: Group A—Control treatment; B—3 times at 5 minutes; C—3 times at 3 minutes; and D—3 times at 1 minute.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph G. Stress of multiple seismic events.

TEST QUESTION

Would you expect the roots and base of the tomato plant that is shaken longer and more often to be larger or smaller than the control? Why, or why not?

VARIATION 3

The Effect of Fertilizer Concentration on Shaken Plants

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will vary the amount of fertilizer among the three experimental plants while shaking them all the same length of time to determine how the young plants are affected.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24:

- Fertilizer solution of concentrations 50%, 100%, 150%, and 200%

HYPOTHESIS GENERATION

Question

Can adding fertilizer make plants grow taller even when they are being shaken?

Sample Hypothesis

If plants receive additional fertilizer while being shaken, their height will increase.

Rationale

The increase in fertilizer will provide extra nutrients for plant growth.

Sample Experimental Procedure

1. Same as Variation 1, but shake plants 1 minute each day.
2. Place 1 shaken plant in each of the following solutions: distilled water and 50%, 100%, 150%, and 200% concentrations of fertilizer solution.
3. Grow 2 or 3 replicates, or have 2 or 3 groups duplicate this variation.

Interpretation

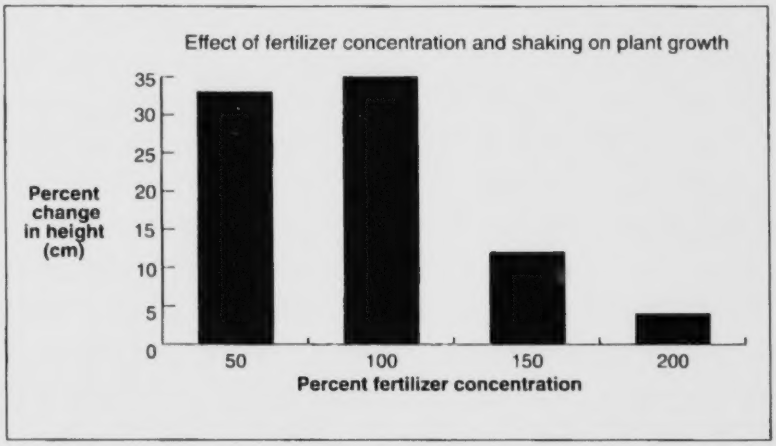
Multiple seismic events of longer duration affect plant height negatively.

Answer to Test Question

The answer should suggest that increasing stress will cause the growth rate to increase in areas of the plant that support the plant structurally.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph H. Fertilizer concentration and shaking on plant growth.

TEST QUESTIONS

1. Would you expect the growth rate of a plant to double with double the amount of fertilizer it normally receives? Why or why not?
2. Will adding more fertilizer to a plant always result in increased growth?
3. Give three examples of components in plant fertilizer and explain how they affect plant growth.

VARIATION 4

Light as a Stressor of Plant Growth

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS

Students will increase exposure of shaken plants to light to determine if the increase in light exposure will overcome the effects of mechanical stress.

HYPOTHESIS GENERATION

Question

What effect will increase in light period have upon growth of plants that are mechanically stressed?

Sample Hypothesis

If plants receive additional light while being shaken, their height will increase.

Rationale

Light will be an additional stressor with heat that will remove needed water from the system. It will have a negative effect upon plant growth.

Sample Experimental Procedure

Same as Variation 1, but maintain the 4 shaken plants at 16, 20, and 24-hour light cycles. Grow 2 or 3 replicates, or have 2 or 3 groups duplicate this variation. The exposure of the control plant should be varied initially to determine whether additional light really does increase plant height and/or mass.

Interpretation

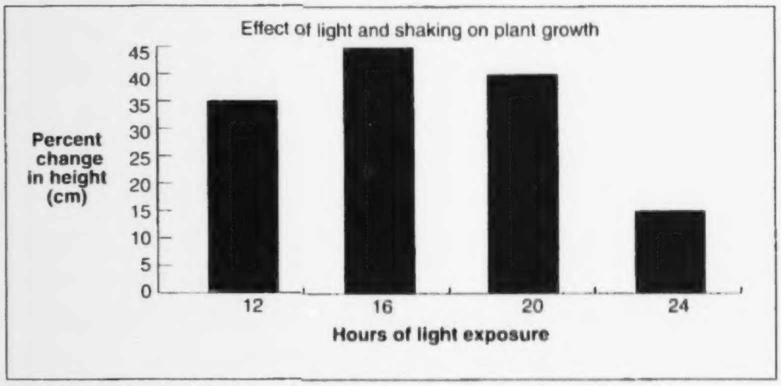
The plants that received 50% fertilizer concentration did as well as plants in the concentration suggested by the manufacturer. Plants with 150% and 200% concentrations did not do well.

Answers to Test Questions

1. No, because the amount of fertilizer that works best for growing a plant is a very precise amount. Doubling that would probably harm the plant.
2. No. Too much fertilizer can poison and even kill a plant.
3. Answers may include nitrogen (protein and nucleic acid synthesis), phosphorus (nucleic acid and phospholipid synthesis; energy metabolism), potassium (enzyme function), calcium (cell wall synthesis), and magnesium (ribosome and enzyme function). [Source: Bidwell, R.G.S. 1979. *Plant Physiology*, 2nd edition. New York: Macmillan Publishing Co., 726]

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph I. Light and shaking on plant growth.

TEST QUESTION

Will giving a plant more light always result in increased growth?

VARIATION 5

The Effect of Thigmic Stress on Plant Growth

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Students will determine how the stress of touching a plant's leaves affects its growth pattern.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 1 5 x 5 x 1-cm piece of foam rubber or sponge

HYPOTHESIS GENERATION

Question

What effect does touching the leaves of a plant have upon their growth?

Sample Hypothesis

Touching plants will stimulate cellular growth and produce leaves with a greater mass than untouched plants.

Rationale

Touching should stimulate growth hormones in the plant, causing an increase in mass.

Sample Experimental Procedure

Instead of shaking, gently brush against the entire surface of the experimental plants with a small piece of foam, fabric, or your hand for 15-second intervals 3 times per day.

Interpretation

Long light cycles negatively affect plant height.

Answer to Test Question

No. Plants are adapted to different levels of light; some grow best in shade, while others grow better in full sunlight.

Interpretation

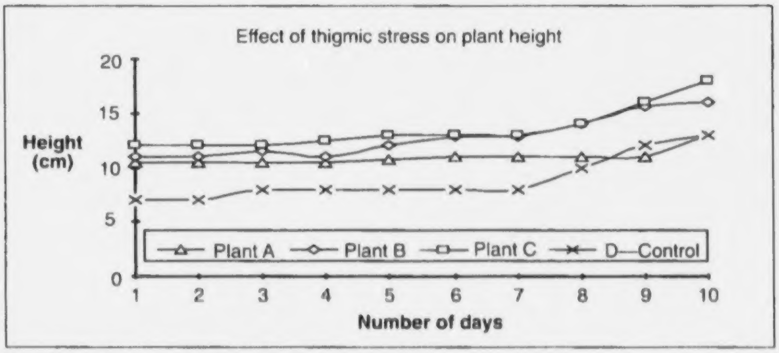
Thigmic stress increases plant height. Leaves tend to increase in length with thigmic stress, but the increase in leaf number varies with the stressor.

Answer to Test Question

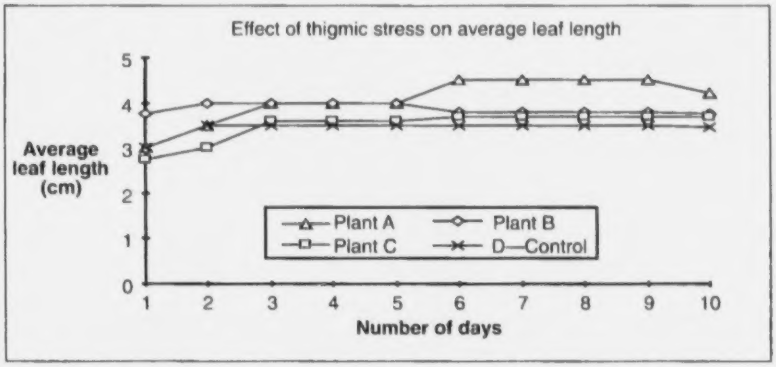
Answer should include a means to measure and compare each plant's response to thigmic stress.

DATA ANALYSIS AND INTERPRETATION

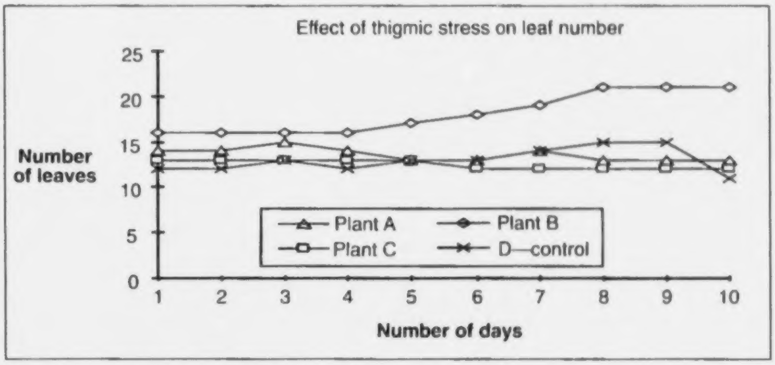
Sample Data



Graph J. Thigmic stress on plant height.



Graph K. Thigmic stress on average leaf length.



Graph L. Thigmic stress on leaf number.

TEST QUESTION

You are a gardener looking for plants to place along a crowded walkway. Design an experiment you could use to choose between two types of plants for this purpose.

VARIATION 6

The Effect of Herbivory Stress

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Insects damage plants by eating the leaves, stems, and roots. Diseases can enter the wounds formed by this damage. Once the cuticle layer has been injured, the plant loses one of its mechanisms of defense.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24:

- 1 hole punch, stapler, scissors, or needle-nosed tweezers

HYPOTHESIS GENERATION

Question

Does the stressor of herbivory affect plant growth?

Sample Hypothesis

If holes are punched in tomato leaves to simulate the stress of insect herbivory produced by feeding, the plants will not grow as tall or have as many leaves.

Rationale

Insect damage resulting from feeding from herbivory will weaken a plant and affect plant growth negatively.

Sample Experimental Procedure

1. Label the plants A, B, C, and D.
2. Form holes in the leaves with a hole punch, stapler, or scissors (see Figure 10) as follows:
   - A: 0
   - B: 1
   - C: 2
   - D: 3
3. Record the height and number of leaves of each plant. Measure the height of the plant from the soil line to highest "V" on the plant. See Figure 11.

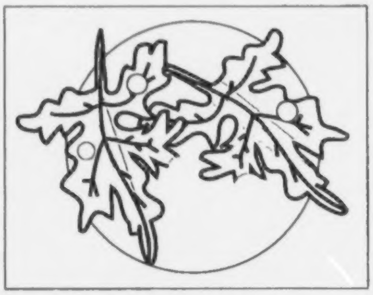


Figure 10. Holes punched in leaves.

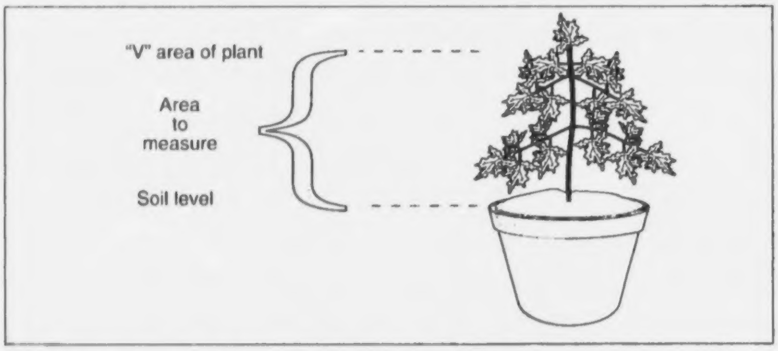
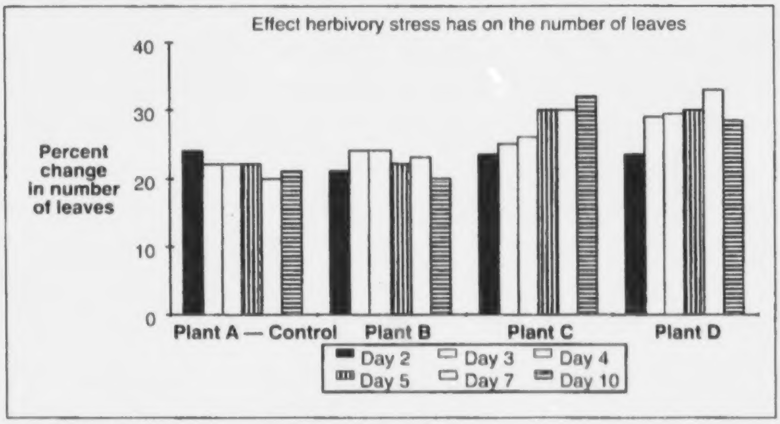


Figure 11. Schematic of area to measure for plant height.

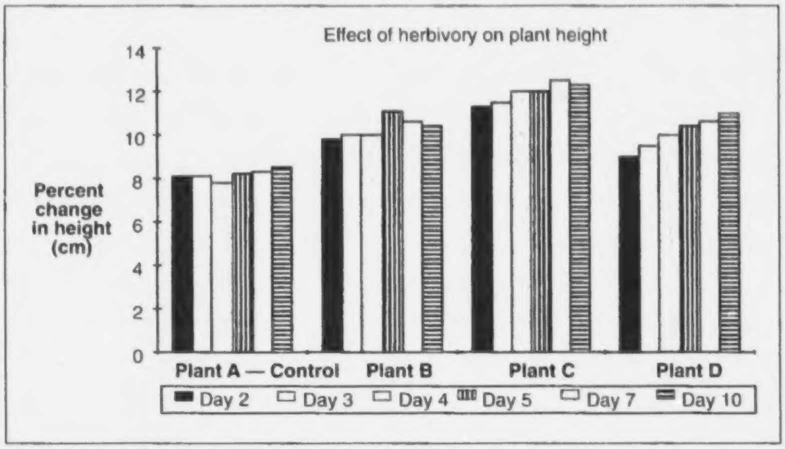
TEACHER'S NOTES

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph M. Herbivory stress on the number of leaves.



Graph N. Herbivory stress on plant height.

Interpretation

Stress from herbivory appears to affect plant height and leaf production positively.

TEST QUESTION

List two ways that insect feeding can damage plant leaves.

Answer to Test Question

Mechanical tissue damage; loss of protective cuticle layer that can cause dehydration and make the plant vulnerable to infection; insects themselves can introduce disease into wound.

VARIATION 7

The Effect of Combined Stressors on Plant Growth

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students examine the effects of the combined stressors of wind and shaking on plant growth.

HYPOTHESIS GENERATION

Question

If the stresses of wind and shaking are combined, do plants grow even less tall?

Sample Hypothesis

Plants that are both blown and shaken will grow less tall than those which receive either stress alone.

Rationale

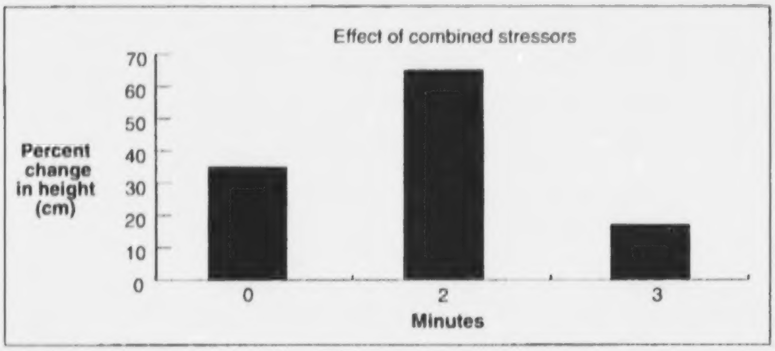
Combined stressors have an additive, negative effect on plant growth.

Sample Experimental Procedure

Same as Variation 1, but add a daily treatment of a 2 to 3-minute period of fan blowing and shaking to the experimental plants.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph O. Change in height with combined stressors.

Interpretation

When the fan and shaking were combined, plant growth with 2 minutes of this treatment increased, while the plants exposed to 3 minutes of this treatment decreased in growth.

TEST QUESTION

Compare and contrast the growth of a tree grown at the ocean shore and a tree grown inland.

Answer to Test Question

Near the ocean, trees would experience a minimum of two stressors—high ocean winds and salt spray. Both would contribute to decreased growth. Although salt and winds would be expected 10 miles inland, the combined salt and daily intensities of the wind would be less. Trees could be expected to be greater in height inland.

VARIATION 8

Pollution as a Plant Stressor

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will examine the effects of a pollutant stressor, such as acid rain, on plant growth.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24:

- 1 bucket
- 1 roll of Hydrion paper range 2 to 8 or 1 pH meter
- 100 mL vinegar
- 100 mL household ammonia or bleach

HYPOTHESIS GENERATION

Question

What effect will the pollutant acid rain have upon plant growth?

Sample Hypothesis

If plants are subjected to varying pH levels of acid rain, then plants exposed to the more acidic rain will have decreased growth rates compared to plants exposed to rain with a normal pH.

Rationale

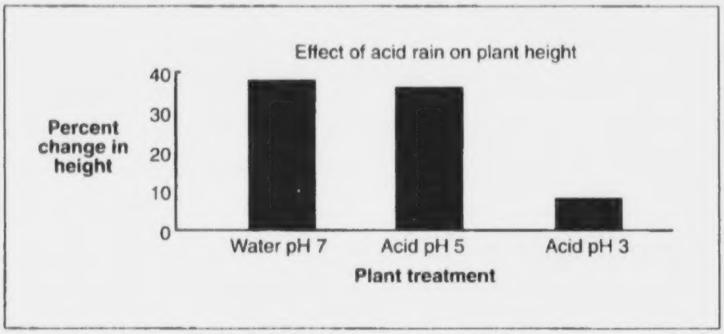
All organisms have an optimum pH for growth. Rain that is not within the optimum range will be injurious to plant growth.

Sample Experimental Procedure

1. Set out containers to collect rainwater.
2. Measure the pH level of the water with a pH meter or paper.
3. Adjust the rainwater solution to the following pH levels: 3, 5, and 7. Vinegar and/or ammonia may be used to adjust the pH level.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph P. Acid rain and plant height.

Interpretation

The height of the plants decreases as the pH level of the rainwater decreases.

TEST QUESTION

Wastewater with a pH of 9 leaks from a factory into one corner of a farmer's corn field. Would you expect the corn plants in that corner to be affected? Explain.

Answer to Test Question

Yes. Normal rainwater has a pH of 6 to 7. An alkaline pH of 9 could stress the corn plants where the leak occurred, causing them to grow less and perhaps lower their seed production.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Allow team members to use the Hydrion paper and report on the acidity.

VARIATION 9

The Effects of Population Density as a Plant Stressor

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS

Students will determine the effects of population density on plant growth.

HYPOTHESIS GENERATION

Question

What effect does population density have on plant growth?

Sample Hypothesis

High density of plants will act as a stressor decreasing plant growth.

Rationale

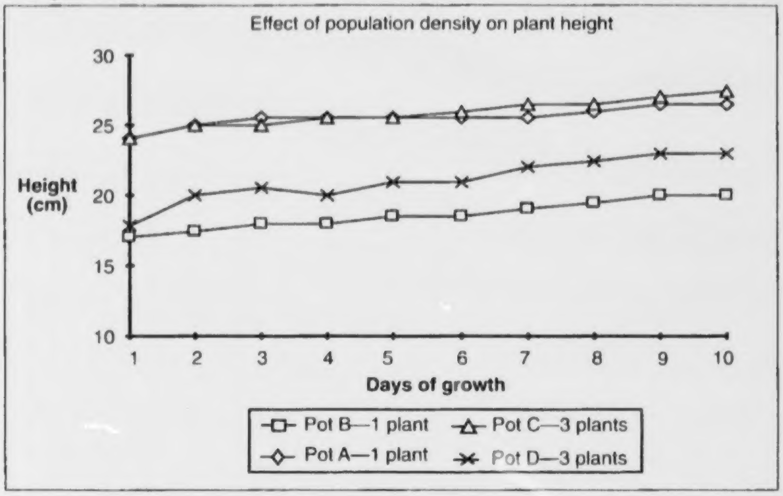
Population density affects competition for resources. Plants with a high population density will have to compete with other populations for available resources. Growth may be lower than in less dense populations.

Sample Experimental Procedure

1. Label 4 pots A, B, C, and D.
2. Plant a single plant in Pots A and B; plant 3 plants in Pots C and D.
3. Record the height of each daily.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph Q. Population density and plant height.

Interpretation

Plants do not appear to be affected significantly by population density. The growth period is only 10 days. This may not be sufficient time to study population density as a plant stressor.

TEST QUESTION

Two oak trees of the same age are cut down for firewood. One grew in the center of an open field, the other in the middle of a dense stand of similar trees. Which tree would provide more wood? Why?

Answer to Test Question

The tree in the open field should provide more wood because it was less crowded—and thus less stressed—so it would have grown more.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Ask students to redesign this experiment. Plant different numbers of seeds in each of six flower pots. Control light, water, fertilizer, and other variables. Compare growth of seedlings.

VARIATION 10

The Effect of Seismic Stress on Plant Germination

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.

SYNOPSIS

The purpose of this experiment is to determine if seismic stress affects the germination of plants.

ADDITIONAL MATERIALS NEEDED

You will need the following for a class of 24:

- 2 plant trays
- 2 kg (4.4 lbs) planting soil
- 1 roll plastic wrap
- 32 bush bean seeds

HYPOTHESIS GENERATION

Question

Will seismic stress inhibit seed germination?

Sample Hypothesis

If seeds are exposed to seismic stress, germination will be inhibited.

Rationale

Seismic stress disturbs developing cells and slows the process of germination.

Sample Experimental Procedure

1. Fill 2 plant trays with planting soil. Label the Trays A and B.
2. Plant 16 seeds in each tray as shown in Figure 12.
3. Moisten and cover with plastic wrap.
4. Place in a warm area.
5. Tray A is the control and is not shaken.
6. Place Tray B on the shaking apparatus for 40 minutes per day.
7. Record observations and measurements for 10 days.

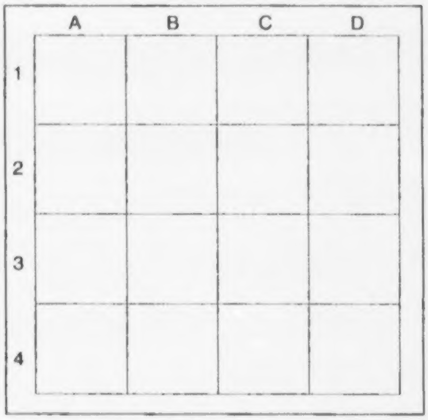
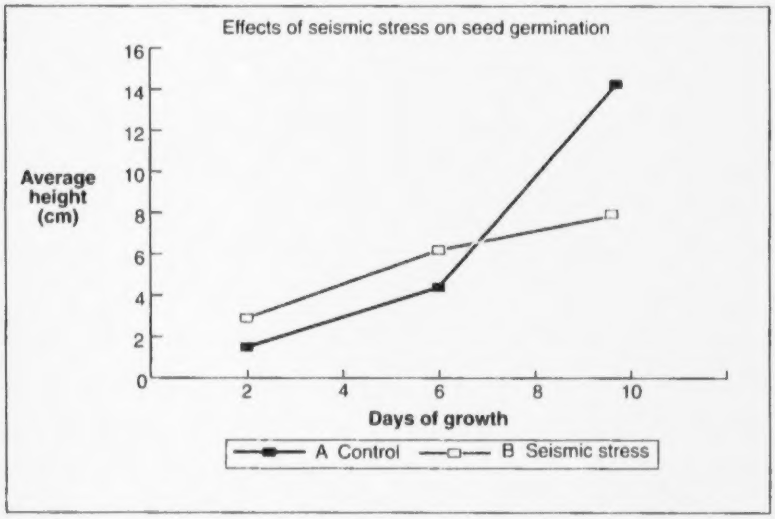


Figure 12. Diagram for planting seeds.

DATA ANALYSIS AND INTERPRETATION

Sample Data



Graph R. Seismic stress and seed germination.

TEST QUESTION

Could seismic stress, such as an earthquake, affect seed germination? Support your answer.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- For students who are blind, provide seeds that are large enough to handle easily to check quality of seedlings.

TEACHER'S NOTES

Interpretation

Seismic stress appears to increase germination height initially, and then slows significantly around the 10th day.

Answer to Test Question

Answers will vary according to student results.

STRESSED OUT PLANTS

DIRECTIONS FOR STUDENTS

INTRODUCTION

What do plants need for optimal growth? Water and nutrients in the soil, proper lighting, and carbon dioxide are all necessary. If any of these are lacking, the plant will be stressed. Much as stress affects humans, different types of stress can affect plants, usually by decreasing their growth. Stress to plants causes billions of dollars in crop losses each year.

Plants also can be stressed by pests, pollution, disease, and temperature. One type of stress that is not often considered is mechanical stress due to wind, precipitation, rubbing, bending, and shaking of plants. See Figure 1. Research has shown that all types of mechanical stress can affect plant growth negatively. Plants may not have feelings, but they do respond to such stresses.

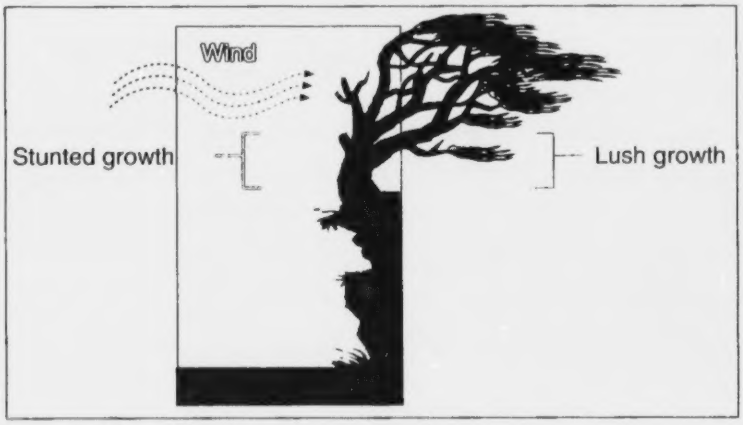


Figure 1. Effects of beach wind on tree growth.

Touching some plants—known as thigmic stress—produces easily visible responses, such as the closing of the Venus Fly Trap or the folding of Sensitive Plant (*Mimosa*) leaves when touched. Seismic stress occurs when a plant is shaken without being touched—by the wind or an earthquake, for example. In this activity, you will examine the effects of plant stressors, such as wind and other environmental conditions.

OBJECTIVES

At the end of this lab, you should be able to:

- Describe the impact of wind stress on plants in different habitats.
- Discuss the effect of wind stress on plant growth.

SAFETY NOTES

Wash hands before and after the lab and after handling the fertilizer solution.

Be careful not to touch hot, delicate fluorescent lights.

Follow all instructions carefully when preparing fertilizer solution.

Alert your teacher to any allergies you may have to certain plants or chemicals.

Alert your teacher if there are any dramatic changes in your plants.

MATERIALS NEEDED

For each team of four students, you will need the following:

- 4 6-cm round pots
- 4 labels
- 100 g potting soil
- 4 tomato plants, 5-cm in height
- 2 spoons
- 1 metric ruler
- 1 50-mL graduated cylinder
- 1 automatic timer
- 1 journal or sketch pad
- 1 marking pen
- 1 spray water bottle
- 1 fan with 3 different speed levels

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of stress on plants. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. Why is it important to control variables?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?
4. What are your independent and dependent variables?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. The effects of loud music on plants is a frequent science fair experiment. How do you think plants would be affected by loud music in your room?
2. How are plants affected by wind vibrations and loud noise at an airport or along a train track?
3. How are plants along sand dunes different from those further inland?
4. How do trees at the top of a mountain differ from those in a valley?
5. When the tall trees are thinned out of a forest, the understory is left unprotected. How would the wind and rain affect these small trees?
6. How would wind blocks affect plant growth?

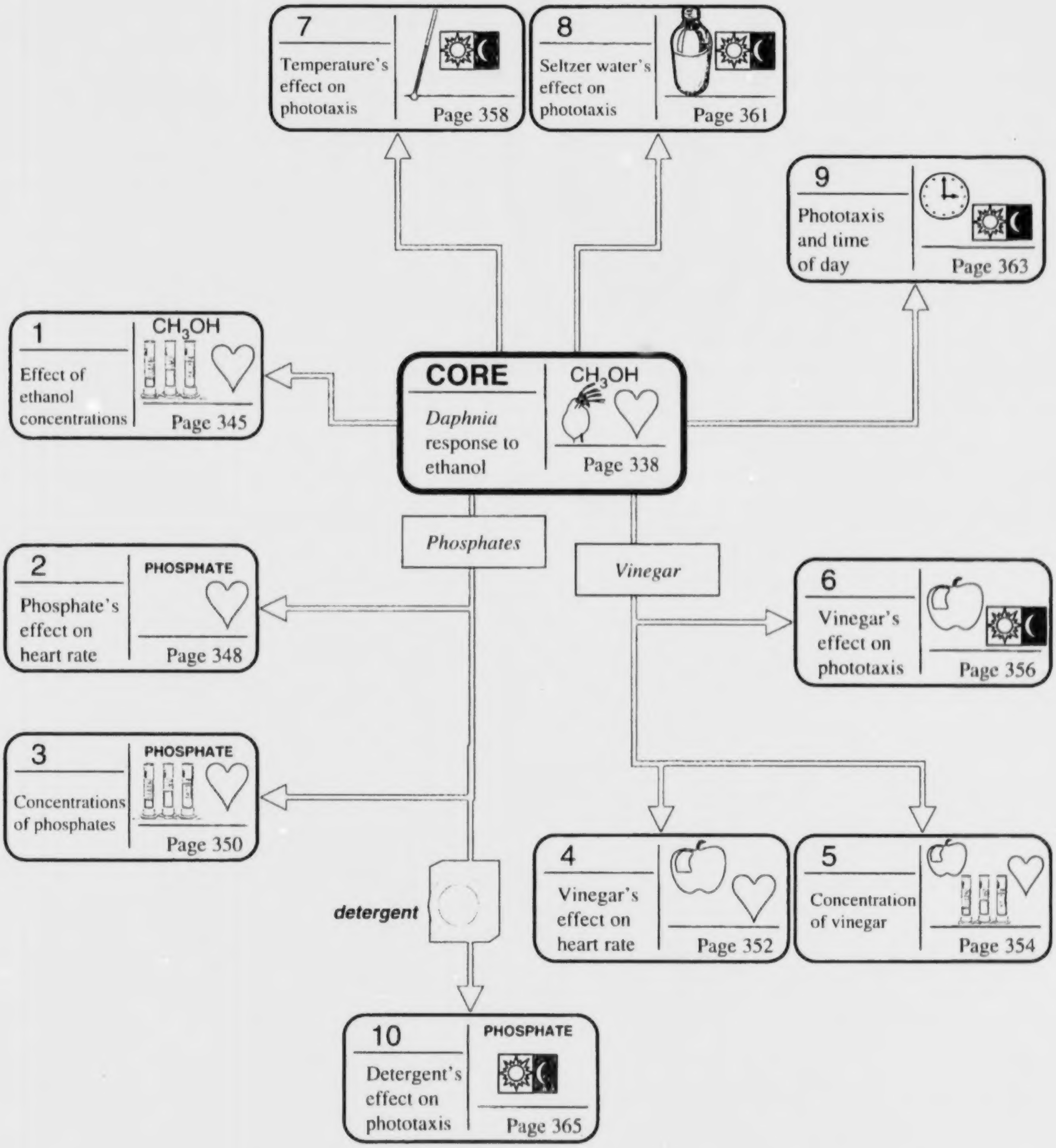
DESIGN OF VARIATIONS OF CORE EXPERIMENT

After collecting and analyzing the data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for experiments you could do next. Think of questions that occurred to you as you conducted the Core Experiment on the effects of wind stress on plant height, mass, and leaf number and average length. What quantifiable experiments could be done based on the observations you made? Questions other students have studied include the following:

- What effect does the mechanical stress of shaking have upon plant growth?
- What effect will increasing periods of seismic stress have upon plant growth?
- Can adding fertilizer make plants grow taller even when they are being shaken?
- What effect will increase in light period have upon growth of plants that are mechanically stressed?
- What effect does touching the leaves of a plant have upon their growth?
- Does the stressor of herbivory affect plant growth?
- If the stresses of wind and shaking are combined, do plants grow even less tall?
- What effect will the pollutant acid rain have upon plant growth?
- What effect does population density have on plant growth?
- Will seismic stress inhibit seed germination?

Nature's Early Warning Systems

At-A-Glance Map of the Core Experiment and Its Variations


7
Temperature's effect on phototaxis
Page 358
8
Seltzer water's effect on phototaxis
Page 361
9
Phototaxis and time of day
Page 363
1
CH_3OH
Effect of ethanol concentrations
Page 345
CORE
CH_3OH
Daphnia response to ethanol
Page 338
Phosphates
Vinegar
2
PHOSPHATE
Phosphate's effect on heart rate
Page 348
6
Vinegar's effect on phototaxis
Page 356
3
PHOSPHATE
Concentrations of phosphates
Page 350
detergent
4
Vinegar's effect on heart rate
Page 352
5
Concentration of vinegar
Page 354
10
PHOSPHATE
Detergent's effect on phototaxis
Page 365

NOTES

Nature's Early Warning Systems

SYNOPSIS FOR CORE EXPERIMENT
Students will predict and test the effects of the ethanol on the heart rate of *Daphnia*.

APPROPRIATE BIOLOGY LEVEL
Introductory or advanced

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Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 345. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Describe possible physiological or behavioral effects of sublethal doses of toxins on *Daphnia*.

MATERIALS NEEDED

You will need the following for a class of 24:

- 1 *Daphnia* culture
- 3 1.4 or 3.8-L (0.5 or 1-gallon) jars
- 1 *Selenastrum capricornutum* culture
- 3 L spring, aquarium, or reconstituted hard water
- 4.0 mL ethanol
- 196.0 mL filtered culture water
- 1 250-mL graduated cylinder or volumetric flask

You will need the following for each group of three students in a class of 24:

- 15 *Daphnia*
- 1 culture dish
- 2 50-mL jars or beakers
- 1 wide-bore pipette
- 2 1-mL plastic, disposable pipettes
- 1 compound microscope
- 1 depression slide and cover slip
- 1 lab journal
- 1 pencil

BEST COPY AVAILABLE

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (50 minutes)

- Explain activity. Draw *Daphnia* from microscopic observations. Practice counting heartbeats.

Day 2 (50 minutes)

- Discuss and form hypothesis. Provide an opportunity to read about *Daphnia*.

Day 3 (50 minutes)

- Observe the heart rate under normal conditions and exposure to ethanol. Analyze and interpret results.

PREPARATION TIME REQUIRED

30 to 40 minutes

- Order *Daphnia*, purchase spring water, and gather materials.

20 minutes

- Set up cultures.

5 minutes

- Prepare the solution of ethanol.

(continued on p. 336)

TEACHER'S NOTES

(continued from p. 335)

- ♥ 1 clock with second hand or a stopwatch
- ♥ 1 calculator
- ♥ several paper towels or filter paper
- ♥ 2 pairs of latex gloves (*optional*)
- ♥ 25.0 mL 2.0% ethanol solution

SAFETY PROCEDURES

Wear goggles and a lab apron when working with chemicals.

Do not use caffeine or nicotine.

Avoid pesticides and insecticides.

Use only substances that can be poured safely down the sink to make disposal easier.

Work away from any open flames when using ethanol.

Prepare the student dilutions in a ventilation hood.

Wear latex gloves when working with ethanol. It can be absorbed through the skin.

Wash your hands after all work is completed.

Do not consume ethanol.

Dispose of broken glass in the proper receptacle.

Use KittyLitter™ to absorb any large spills. Sweep up the KittyLitter™.

DIRECTIONS FOR SETTING UP THE LAB

- Glassware should be cleaned with hot water and rinsed thoroughly.
- Prepare the 2.0% ethanol solution by combining 4.0 mL ethanol and 196.0 mL filtered culture water in a graduated cylinder or volumetric flask.

TEACHER BACKGROUND

Content Information

Daphnia, commonly called water fleas, are not insects, but small freshwater crustaceans (Class Crustacea, Subclass Brachiopoda, Order Diplostraca, Suborder Cladocera). They are an important source of food for young fish. A transparent exoskeleton covers the entire body. Through it, one can see eggs in a female's brood pouch hatch and develop. While still in the brood pouch their paired lateral eyes combine, forming the large single eye characteristic of adults. The light-colored organ lying above the eye is the brain. Two pairs of antennae protrude from the head. The smaller pair bears sensory organs. The larger, branched pair of antennae is used as oars to move the water flea up and down. It cannot move forward or backward (Kramer, 1987).

Inside the exoskeleton are five pairs of legs with comb-like gills attached to them. When the legs kick forward, they bring a stream of water across the gills and wash bits of food up to the mouth that lies just beneath the beak. The preferred food for *Daphnia* is bacteria, but for most species it is unicellular algae (Weber, 1991). The food travels from the mouth, through the esophagus, to the stomach that connects to the intestine. The dorsal heart is clearly visible even though the blood cells are colorless. In females, a large brood chamber that usually contains eggs ((2 to 40) is posterior to the heart. As the eggs develop they become less distinct. Secretions from the walls of the brood pouch nourish the developing *Daphnia*. See Figure 1.

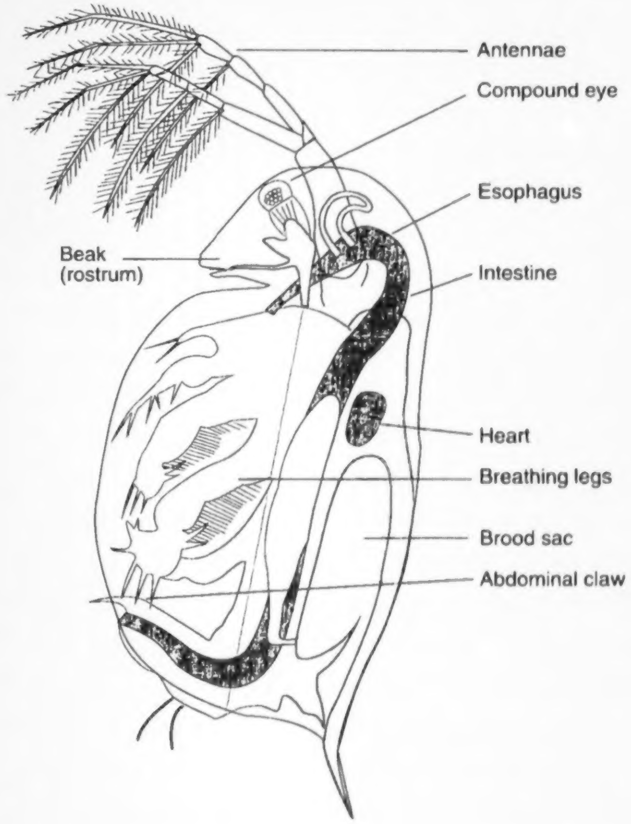

Figure 1. A species of *Daphnia* with several internal structures identified. (Used with permission. Collins, K. & B. (1985). *Daphnia* — A Handy Guide. *The American Biology Teacher, 47*(50), 299.

Only female *Daphnia* can be found in nature, during the spring and summer. These females reproduce parthenogenetically when they are about 10 days old, producing offspring that are identical to themselves. Later in the year, in response to changes in temperature, light and food availability, small males are produced also. Sexual reproduction produces fewer, but larger eggs. These eggs contain more yolk and thicker shells that nourish and protect the offspring until conditions improve. As further protection from a harsh environment, the walls of the female's brood pouch also thicken. When she molts, the walls remain around the eggs.

Daphnia have many uses in the laboratory. Their transparent covering allows their physiology to be studied while they are alive. They also may be used as food for hydra or aquarium fish. They represent an important link in many freshwater ecosystems. The stomach contents of young fish are rarely less than 10% *Daphnia* and may be as much as 95% *Daphnia* (Pennak, 1989). Indicator species serve as an early warning that a community or an ecosystem is being degraded. Since the 1940's, *Daphnia* have been used as an indicator species in lab tests for toxicity thresholds of industrial wastes. They are more sensitive than trout, a fish known for its susceptibility to poisons. Standards for using them in detecting toxic materials in freshwater are established by the Environmental Protection Agency (EPA) (Weber, 1991). Those

TEACHER'S NOTES

guidelines even specify the appropriate food to give animals used in toxicity tests. For example, *Daphnia* fed diatoms are more tolerant to pollutants than those fed green algae alone.

A chemical may be beneficial, toxic, or lethal to *Daphnia*. A chemical can be beneficial if the exposure is moderate and the chemical is used under the proper circumstances. Under certain conditions, it can be toxic: causing harm, but not killing the *Daphnia*. A chemical is lethal if it kills the *Daphnia*. Factors that affect whether a chemical is beneficial, toxic, or lethal include how it enters the body, the body weight, exposure time, and the amount of chemical. A chemical may be very toxic in a low dosage, but lethal to the same individual at a higher dosage.

Like many planktonic organisms, *Daphnia* migrate up and down. They usually are found actively swimming in the early morning and late afternoon when the sun is low, but have inconspicuously retreated to the bottom of a shallow lake in the middle of the day. Experimentally blinded animals still migrate (Pennak, 1989). Is there a light sensor somewhere else in the body? Is the rhythm endogenous? Can disruptions of this behavior occur if sublethal amounts of toxins are in the environment? At least the last of these questions has been addressed. Goodrich and Lech (1990) developed an assay to assess the disruption of spatial orientation of *Daphnia magna*.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts:

Correct Concept	Misconception
• Low levels of toxins can affect organisms.	• It takes high levels of toxins to affect organisms.
• Clear water may contain toxins.	• Clear water is free of chemical toxins.
• Indicator species as small as *Daphnia* serve as an early warning that a community or an ecosystem is being degraded.	• Only large, complex organisms are good indicator species for observing the effects of pollution.
	• Indicator species serve as early warnings that a community or an ecosystem is being depleted.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

The teacher should begin the Core Experiment by giving each laboratory group a flask or culture dish containing *Daphnia*. As the students observe the specimens, the teacher can prompt a discussion with the following questions:

- Are all of the organisms of the same species or different species?
- Compare and contrast the organisms' size, swimming motion, number of appendages, and degree of transparency.
- If they are the same species, what inferences might be made to explain the differences in size?

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

TEACHING TIPS

- When transferring *Daphnia* from one container to another, never pour the liquid through the air. Air may become trapped under their carapaces, causing them to float. They are unable to break the surface tension of the water and will be trapped above the water surface and die. Instead, keep the pipette tip submerged.
- If *Daphnia* are to be used more than a couple of days, feed them with a non-filamentous alga such as *Selenastrum capricornutum*. Biological supply companies also sell a concentrated food.
- A 3-L culture of 30 animals should provide about 300 young *Daphnia* per week (Weber, 1991).
- At 28°C, *Daphnia* live an average of 26 days; at 18°C, 42 days; and at 8°C, 108 days (Pennak, 1989).
- Aerate the culture lightly and provide a light cycle of 16 hours light/8 hours dark. Low oxygen concentrations are well tolerated, so you may not need to aerate.
- At weekly intervals, siphon off and discard 90% of the culture medium. Cover the end of the siphon tube with fine mesh, such as organza, to avoid removing *Daphnia*. Gently pour the remaining 10% of the medium with animals into fresh culture medium. The tube may be made from glass tubing, a Pasteur pipette, or plastic transfer pipette.
- Spring water is obtained easily from supermarkets or from biological supply houses. You may have greater survival with aquarium water or with reconstituted hard water (Weber, 1991).
- Use a dishwasher to clean all glassware if one is easily accessible within the school.
- Glass should be cleaned between each use.
- Sublethal solutions of toxins should be measured in terms of the percent of solute in the solvent (weight/volume or volume/volume).
- Encourage your students to think of conditions they might need to control in measuring heart rate. Consider how the temperature and light might change if they leave their slides on the microscope stage with the light.

(continued on p. 339)

After observing the cultures, have students read "Canaries of the Stream" (Essman & Zarpas, 1990). Follow the reading with a discussion of chemical pollution in our streams and rivers by industry, farms, cities, and individuals. Bring in examples of local industries and other sources. Lawn care products and automobile maintenance wastes are sources of pollution in even the most clean industrial area. Companies whose products involve making or using glues or resins are likely to have the potential to discharge waste alcohol. Ask the following questions:

- What activities did you observe in your *Daphnia*?
- Do you expect the activities to change if the environment of the *Daphnia* changes?
- How could you measure or quantify those activities?
- What environmental changes might occur in a lake or pond as a result of human activity?
- What environmental changes occur in a lake or pond even without human activity?

Sample Hypotheses

- The heart rate of *Daphnia* will decrease when the culture is enriched with lawn fertilizer.
- The exposure of *Daphnia* to rain run-off from the school parking lot will cause a reduction in the population growth.
- Exposure to ethanol will result in a decrease in the percentage of *Daphnia* showing positive phototaxis.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 343.

Question

What is the effect of ethanol on the *Daphnia* heart rate?

Hypothesis

Exposure to ethanol will cause a decrease in the *Daphnia* heart rate.

Rationale

Ethanol depresses the heart rate in humans and may have the same effect on *Daphnia.*

Procedure

1. Obtain 15 *Daphnia* with a wide bore pipette. Place in a 50-mL jar filled with culture water.
2. Set up a second jar or culture dish with culture water to act as a recovery area for treated *Daphnia*.
3. Prepare a wet mount of an adult *Daphnia* using culture water. Use a depression slide to avoid crushing the animal. Pieces of a broken cover glass or a ring of gel may be used to support the cover slip.
4. Observe the *Daphnia* under low power. Identify the following structures: heart, brood sac, swimmers, beak, eye, and antennae. Note the location of the heart and its rate.

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TEACHING TIPS

(continued from p. 338)

- Many factors may influence the heart rate:
  - The heart rate may increase after feeding (Baylor, 1942 in Maynard, 1960).
  - Light may increase the heart rate for animals kept in dim light (Schulz, 1928 in Maynard, 1960).
  - Circadian rhythms influence the heart rate. *Daphnia*'s heart rate is greatest in late afternoon and early evening (Tonolli, 1947 in Maynard, 1960).
  - Some materials in the environment will not affect the heart rate, but if they are in the circulatory system they will affect it (Lévy, 1927 in Maynard, 1960).
  - Increasing temperature, over the normal environmental range, increases the rate of heart contractions about 50 at 5°C to about 200 at 27°C (MacArthur & Baillie, 1929 in Maynard, 1960).
- A constant temperature is necessary to ensure consistent experimental results. For standard toxicity assays, the culture temperature should be 20 ± 2°C (Weber, 1991), but normal growth will occur from 18 to 26°C.
- If you want your students to appreciate the probabilistic nature of science, encourage them to analyze their results statistically. For paired sets of data, a Sign Test or *t*-test of Paired Comparisons is appropriate as is the Wilcoxon's Signed-Rank Test. The latter is explained in the analysis of the Core Experiment. For all of these tests, the tabled values for comparison should be considered a black box; simply use the values without concern for their derivation.

TEACHER'S NOTES

(continued from p. 339)

5. Sketch the *Daphnia* using these observations.
6. After 20 minutes of observation, select a team number assignment from 1 through 10.
7. Student Teams 6 through 10 should go to Step 8. Student Teams 1 through 5 should do the following procedure:
   a. Remove the cover slip and use a paper towel or filter paper to remove all the culture water carefully from under the cover slip of the depression slide well. Alternatively, use a disposable, plastic 1-mL pipette to remove a majority of the culture water from the depression slide so that area of movement of the *Daphnia* is reduced significantly. This will improve the process of counting heart beats.

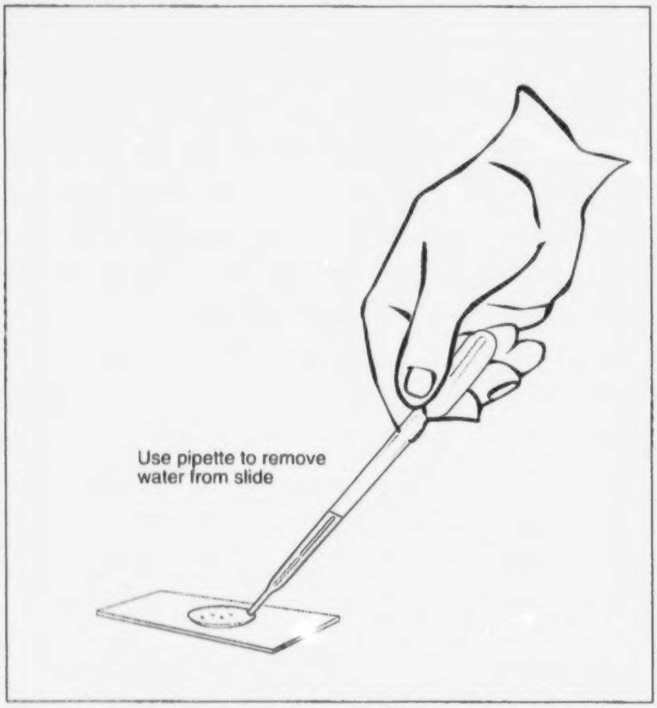

Use pipette to remove water from slide

Figure 2. Removing water from *Daphnia* in a depression slide.

   b. Count the number of heartbeats in 15 seconds with a calculator as follows:
      Step 1: The student who will be counting the heartbeats should enter 1 + 1 on the calculator and focus on the *Daphnia's* heart.
      Step 2: At the other partner's signal, the student observing the heart rate should count heartbeats by tapping the "=" key on the calculator for each heartbeat observed.
      Step 3: After the 15-second signal provided by the partner, the student observing the heartbeats should stop entering heartbeats on the calculator.
      Step 4: Calculate the number of heartbeats per minute by subtracting the additional "1" that was entered initially and multiply this value by 4. For example, if the calculator reads 84 beats at the end of the 15-second interval, subtract "1" for a value of 83. Multiply this value by 4 for the number of heartbeats per minute: 332.

(continued on p. 341)

(continued from p. 340)

c. Use a second 1-mL disposable pipette to replace the water with enough 2.0% ethanol solution to submerge the *Daphnia*.

d. Count the *Daphnia* heartbeats at 2-minute intervals over a 10-minute period as described in 7b, Steps 1 to 4. Team 1 should observe their *Daphnia* at 2 minutes; Team 2 at 4 minutes; Team 3 at 6 minutes; Team 4 at 8 minutes; and Team 5 at 10 minutes.

e. After the count is made, quickly remove the alcohol with the disposable plastic pipette. Exposure time of each specimen should be minimized where possible. Return the specimen to a culture container reserved for specimens that already have been subjected to the alcohol treatment. This will eliminate the chance of using a specimen more than once and reduce the risk of contaminating the culture with residual ethanol.

8. Student Teams 6 through 10 should do the following procedure:

a. Remove the cover slip and use a paper towel or filter paper to remove all the culture water carefully from under the cover slip of the depression slide well. Alternatively, use a disposable, plastic 1-mL pipette to remove a majority of the culture water from the depression slide so that area of movement of the *Daphnia* is significantly reduced to improve the process of counting heartbeats.

b. Replace the water with enough 2.0% ethanol solution to submerge the *Daphnia*.

c. Observe the *Daphnia* heartbeat at 2-minute intervals over a 10-minute period as described in Steps 7 a to c. Team 6 should observe their *Daphnia* at 2 minutes; Team 7 at 4 minutes; Team 8 at 6 minutes; Team 9 at 8 minutes; and Team 10 at 10 minutes.

d. After the count is made, quickly remove the ethanol from the slide. Replace it with a drop of culture water. Exposure time of each specimen to the ethanol should be minimized.

e. After 5 minutes in the culture water solution, count the number of heartbeats in 15 seconds and record the heart rate of the specimen in beats per minute. Return the specimen to a culture container reserved for specimens that have been used already to eliminate the chance of using a specimen more than once and reduce the risk of contaminating the culture with residual ethanol.

f. Pool class data for analysis.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 1. Effect of 2.0% ethanol on the heart rate of *Daphnia*.

Student team	Untreated (beats/minute)	Ethanol exposed (beats/minute)	Retreated with culture water (beats/ minute)
1	296	184	
2	312	221	
3	288	133	
4	336	121	
5	296	65	
6	252	156	224
7	220	155	198
8	244	112	225
9	252	91	150
10	248	54	98

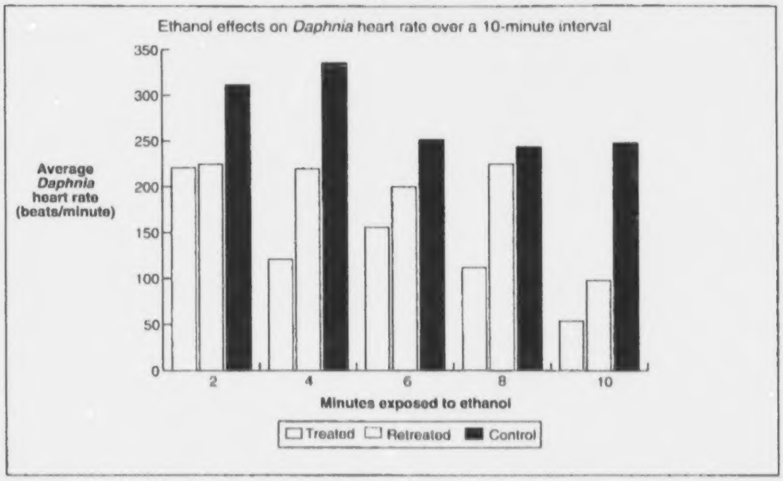
TEACHER'S NOTES

Interpretation

These data support the hypothesis that ethanol will cause a decrease in the heart rate of *Daphnia*. The heart rate of *Daphnia* exposed to ethanol over a 10-minute interval was depressed by 78%. At a concentration of 2.0%, *Daphnia* detect ethanol as a toxin.

Answer to Test Question

Dividing the sample removes the variable of handling introduced by distributing the effect of handling equally between the ethanol-exposed and unexposed specimens, leaving only exposure as the variable to evaluate.



Graph A. *Daphnia* heart rate before and after exposure to 2.0% ethanol, and recovery period.

TEST QUESTION

Why must half the *Daphnia* be evaluated first with ethanol and the other half evaluated first with culture water?

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted their first experiments. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- Are there physiological or behavioral effects that you observed that might be a result of the low levels of toxin?
- Was time a factor?
- What is the value of indicator species such as *Daphnia*?
- If *Daphnia* is a good indicator organism for low levels of ethanol, to what else might it be sensitive?
- Is there a level at which you can kill the indicator species?
- What might be the long term effects of sublethal doses of toxins?
- How might sublethal doses affect longevity?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

- Provide a complete copy of the laboratory experiment printed in braille. An enlarged diagram of the structures of *Daphnia* (Figure 1) should be included as a raised-line drawing.
- Encourage the visually impaired student to record lab data in braille and make a typewritten copy for the team. A computer enables a visually impaired student to act as a recorder for the team.
- Ask the visually impaired student to provide a typed lab report and to keep a braille copy for personal use.
- Ask the visually impaired student to make graphs of data collected to accompany the team lab report.

Deaf or Hard-of-Hearing

- Allow hearing impaired students to participate fully in the experiment.

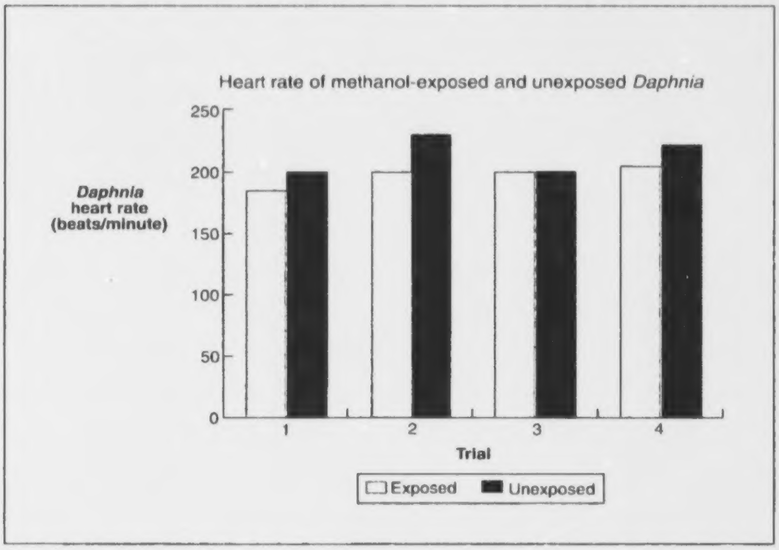
Mobility Impaired

- Provide a lab table with a height that accommodates wheelchair access.
- Permit wheelchair movement if the classroom is large enough to allow the mobility impaired student to obtain supplies as needed.
- Have lab team members prepare slides for the manually impaired student to observe and count the heartbeat of *Daphnia.*
- Provide the manually impaired student with a tape recorder or other means to collect data and to take notes.
- Provide the manually impaired student with a microscope equipped with levers that can focus the fine adjustment.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. Ethanol, the alcohol in liquor, is a depressant for humans. If methanol, a poison for humans, is a depressant for offspring, do these data graphed below support your expectation? Explain.



Graph B. Influence of methanol on heart rate of *Daphnia.*

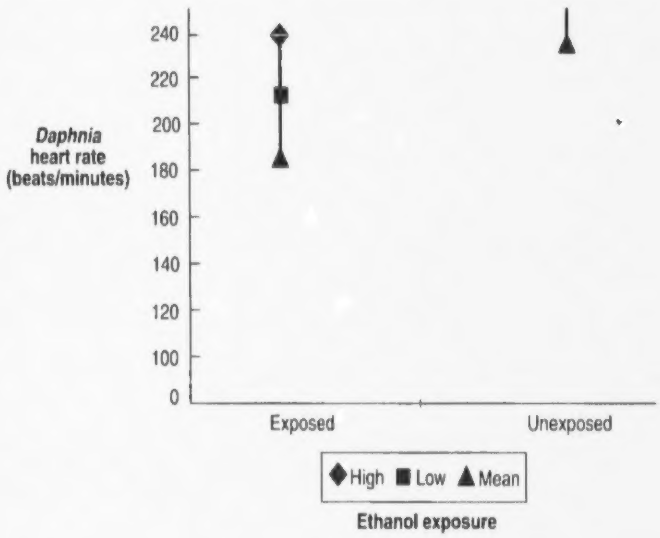
2. Why was it desirable to pool class data to evaluate the effect of ethanol on the heart rate of water fleas?

Answers to Additional Test Questions

1. The expectation was that the methanol would depress or lower the heart rate of water fleas, or maybe even kill them. For three of the four tested animals, the heart rate was lowered and for the fourth there was no change. This supports the hypothesis. The number of water fleas examined is too small to dismiss the possibility that chance alone accounts for these observations.
2. Many factors can influence the responses of an organism. Here, there was no control over or no way to measure the age of the animal, the amount of heat supplied by the microscope light, the oxygen deprivation provided by reduced water, or handling stress. Since these, and possibly other factors, could provide variations in the response, several samples were needed to obtain a measure of the true response.

Answers to Questions and Analysis on Student Page

1. See Graph C for a representative graph.
2. These data support the hypothesis that ethanol would depress the heart rate of *Daphnia*, but there is an overlap in the values. It would be appropriate to analyze these data statistically as well as display them graphically.
3. If half of the animals had depressed heart rates and half had elevated heart rates, reject the hypothesis. Results like these are the same as the results one would expect if a coin were tossed. Half the time one would expect heads and half the time one would expect tails.
4. If the animals showed a depression consistently greater than that shown by another lab, one would question whether the techniques were consistently different. If the exact same procedure was followed and the animals were measured at the same time of day, a consideration may be that the animals differ genetically.
5. An easy way to test whether the animals are different would be to exchange animals and retest. A possible hypothesis is that no difference in the depression of heart rate between the two cultures would be found. Some of each original culture should be retained.



Graph C. Pooled class data on ethanol's effect on *Daphnia* heart rate.

POSSIBLE SOURCES OF MENTORS

- Local college or university toxicology professor.
- Local research or industry that deals with toxicology.

REFERENCES AND SUGGESTED READINGS

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VARIATIONS OF CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

VARIATION 1

The Effect of Different Concentrations of Ethanol on the Heart Rate of *Daphnia*

SYNOPSIS

Students will compare the heart rates of *Daphnia* exposed to different concentrations of ethanol.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- 5 mL 8.0% solution of ethanol
- 5 mL 4.0% solution of ethanol
- 5 mL 2.0% solution of ethanol
- 5 mL 1.0% solution of ethanol
- 10 to 12 *Daphnia*

BEST COPY AVAILABLE

TEACHING TIPS

- Provide the students with test tubes or small beakers and a syringe or graduated pipette and have them prepare their own dilutions from the 8.0% solution you provide.
- There is a lot of counting and there are several wait periods for the water fleas to adjust to their surroundings. Two or three teams might be encouraged to cooperate on an exercise as demanding as this. The teams could reciprocate by helping one another on another variation they propose. This kind of cooperation is common in large research labs.
- Remind students that the pond water and 2.0% ethanol measurements of another day are better repeated under the conditions of this day.

TEACHER'S NOTES

SAFETY PROCEDURES

- Do not consume ethanol.
- Wear goggles and apron when working with ethanol.
- Work away from any open flames when using ethanol.
- Wear latex gloves when working with ethanol. It can be absorbed through your skin.
- Wash your hands after all work is completed.
- Broken glassware presents a hazard. Dispose of broken glassware in appropriate containers.
- Put KittyLitter™ on any large spills. Sweep up KittyLitter™ after it has absorbed the spilled material.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Prepare the 8.0% ethanol solution by diluting 8.0 mL of ethanol with 92.0 mL of culture water.
- A 4.0% solution is made by diluting 50.0 mL of the 8.0% solution with 50.0 mL of culture water.
- A 2.0% solution is made by diluting 50.0 mL of the 4.0% solution with 50.0 mL of culture water.
- A 1.0% solution is made by diluting 50.0 mL of the 2.0% solution with 50.0 mL of culture water.

HYPOTHESIS GENERATION

Question

What is the effect of varying concentrations of ethanol on *Daphnia* heart rate?

Sample Hypothesis

The higher the concentration of ethanol, the greater the decrease in *Daphnia* heart rate.

Rationale

If some is harmful, more might be even more harmful. The model for humans is that ethanol in large quantities is lethal.

Sample Experimental Procedure

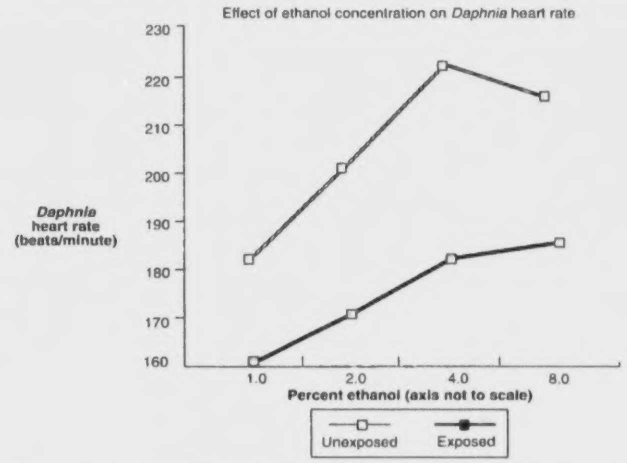
1. Repeat the Core Experiment using these 4 concentrations of ethanol and unadulterated culture water.
2. Expose the *Daphnia* to various concentrations for 5-minute intervals. A the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 2. Effect of ethanol concentration on *Daphnia* heart rate. Values reported are the average rates for 10 animals.

Percent ethanol	Exposed	Unexposed
1.0	160	184
2.0	170	200
4.0	184	224
8.0	186	216



Graph D. Effect of varying ethanol concentrations on *Daphnia* heartbeat rate per minute.

TEST QUESTION

How could you determine if the results of your experiment represent differences in diffusion rates resulting from using solutions of different concentrations?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide syringes or plastic graduated cylinders in a variety of sizes to allow the visually impaired student to prepare the ethanol solutions indicated in the lab.

Interpretation

These data show a slight increase of 13% at 1.0% ethanol to 18% at 4.0% ethanol. These three concentrations of 1.0%, 2.0%, and 4.0% support the hypothesis that increasing concentrations of ethanol will result in increasing depressions of heart rate. The depression of heart rate was less with exposure to 8.0% ethanol than it was with exposure to 4.0% ethanol. These trials were done sequentially and early in the day with the 8.0% observations made first. It may be that some daily rhythm in the water fleas has confounded the results. It is recommended that this exercise be repeated randomizing the treatments.

Answer to Test Question

One would expect that the greater the difference in the concentration of solutions of the internal and external environments of the water flea, the greater the diffusion will be and the more quickly the effect will be detected. With the exception of the most concentrated solution, that is what was found. While the results are consistent with the effects of diffusion, they do not prove that the observed differences are caused by diffusion. It could be that water fleas are able to detoxify ethanol. One could test this by leaving them in a dilute ethanol solution for a longer time. If they are able to detoxify the ethanol, the extended time will not result in their death.

VARIATION 2

The Effect of Phosphate on the Heart Rate of Daphnia

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will compare the heart rate of *Daphnia* before and after exposure to phosphate.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

♥ 2.0% trisodium phosphate (TSP)

SAFETY PROCEDURES

Do not ingest TSP. It may cause burns to eyes and skin.

Wear goggles, gloves, and a long-sleeved garment.

Do not allow the solution to be absorbed through gloves or clothing.

Keep TSP away from eyes.

Wash all contaminated clothing thoroughly before use.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

To prepare a 2.0% phosphate solution, dissolve 2 grams of TSP in a small quantity of culture water and use culture water to dilute the resulting solution to 100 mL.

HYPOTHESIS GENERATION

Question

What is the effect of phosphate on *Daphnia* heart rate?

Sample Hypothesis

The heart rate of *Daphnia* will be decreased if they are exposed to phosphate.

Rationale

Phosphates have been reduced in cleaning products because phosphate enrichment of water causes algal blooms and subsequent loss of aquatic animals. Phosphates may cause problems for some aquatic animals directly.

Sample Experimental Procedure

1. Repeat the Core Experiment using a 2.0% solution of phosphate instead of 2.0% ethanol.
2. Expose the *Daphnia* to various concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

TEACHING TIPS

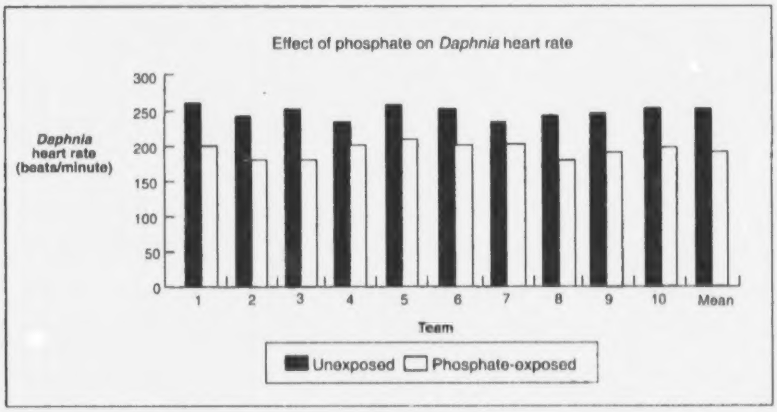
- Although automatic dishwashing detergents contain phosphates, they also contain many other potentially toxic materials, such as chlorine bleach. They should not be used.
- Trisodium phosphate (TSP) is available at hardware stores and may be used. It is a recommended cleaner for surfaces to be painted. You may substitute sodium orthophosphate from the chemical storeroom, but students should know that the phosphate should be something the water fleas likely would encounter in the environment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 3. Results of phosphate exposure on *Daphnia* heart rate.

Student team	Unexposed *Daphnia* (beats/minute)	Phosphate-exposed *Daphnia* (beats/minute)
1	262	200
2	248	180
3	256	180
4	240	200
5	260	212
6	252	200
7	240	200
8	244	180
9	248	192
10	252	196
Mean	250	194



Graph E. Effect of phosphate on *Daphnia* heart rate.

TEST QUESTION

The graph on page 350 displays data collected by another group of students. Interpret their results.

Interpretation

The mean number of beats per minute was 22% lower in the phosphate-exposed animals compared with the unexposed animals. These data support the hypothesis that phosphate pollution will depress the heart rate of water fleas.

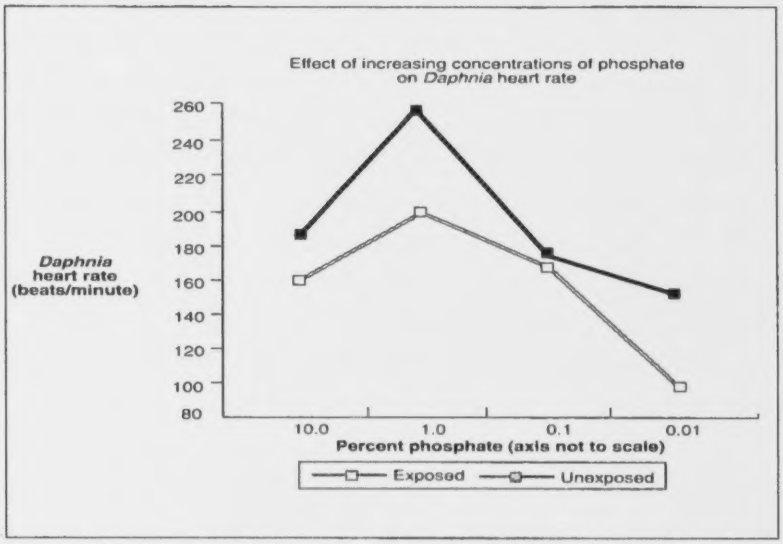
Answer to Test Question

These students found that the heart rates of water fleas increased when they were exposed to phosphate. This could represent a dying response or an initial response that will be reduced later. One needs to know whether these students used the same procedure as others who did this variation. If they did, one would suspect that they did not use the same kind of water fleas or that they used a different source for their phosphate amendment.

TEACHER'S NOTES

TEACHING TIP

Provide students with syringes or pipettes, several test tubes or small beakers, and an 8.0% phosphate solution. Allow them to prepare their own dilutions.



Graph F. Influence of phosphate on *Daphnia* heart rate.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1. Use chemicals specified for Variation 2.

VARIATION 3

The Effect of Different Concentrations of Phosphate on the Heart Rate of Daphnia

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will compare the heart rates of *Daphnia* exposed to different concentrations of phosphate.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- 8.0% solution of phosphate
- 4.0% solution of phosphate
- 2.0% solution of phosphate
- 1.0% solution of phosphate
- 10 *Daphnia*

SAFETY PROCEDURES

See Variation 2.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Prepare the 8.0% phosphate solution by dissolving 8.0 g of phosphate (trisodium phosphate, TSP) in a small amount of culture water and dilute the resulting solution to 100.0 mL.
- A 4.0% solution is made by diluting 50.0 mL of the 8.0% solution with 50.0 mL of culture water.
- A 2.0% solution is made by diluting 50.0 mL of the 4.0% solution with 50.0 mL of culture water.
- A 1.0% solution is made by diluting 50.0 mL of the 2.0% solution with 50.0 mL of culture water.

HYPOTHESIS GENERATION

Question

What is the effect of varying concentrations of phosphate on *Daphnia* heart rate?

Sample Hypothesis

The higher the concentration of phosphate, the greater the decrease in *Daphnia* heart rate.

Rationale

If some is harmful, more should be even more harmful. A decrease in the heart rate may signal impending death.

Sample Experimental Procedure

1. Repeat the Core Experiment using these 4 concentrations of phosphate and unadulterated culture water.
2. Expose the *Daphnia* to various concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 4. Effect of increasing phosphate concentrations on *Daphnia* heart rate. Values reported are the average rates of 10 animals.

Percent phosphate	Exposed	Unexposed
1.0	160	184
2.0	227	250
4.0	170	174
8.0	92	153

TEACHER'S NOTES

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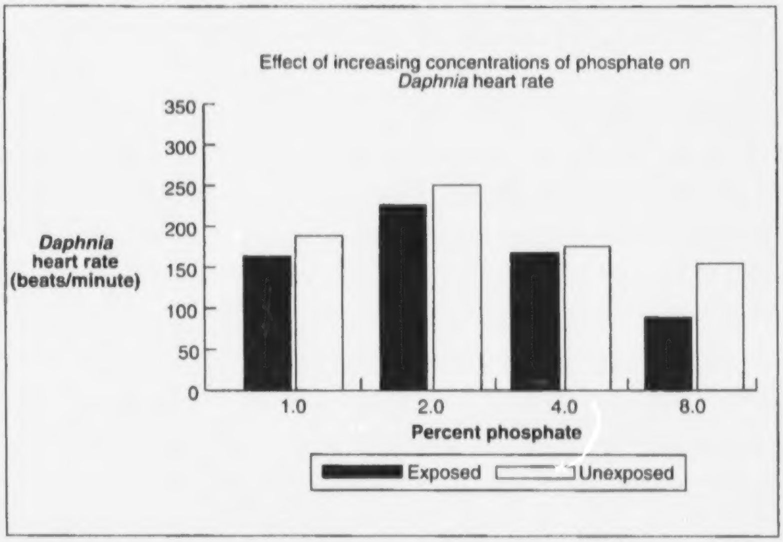


Graph G. Effect of increasing concentrations of phosphate on *Daphnia* heart rate.

Interpretation

These data show irregular changes in heart rate. The greatest depression (40%) occurs with the highest phosphate concentration, but the next greatest depression (13%) occurs at the lowest concentration. If these were the only 2 concentrations tested, one would conclude that these data support the hypothesis. Here, however, less depression in the heart rate was found at 2.0% phosphate (9%) and 4.0% phosphate (2%) than at the extremes tested. Perhaps the other team that collected data did not follow the same procedure exactly. The 5-minute adjustment period could be crucial for obtaining repeatable results.

TEST QUESTION

Over a period of 2 to 3 weeks, an increase in phosphate concentration in a pond or lake initially can cause an increase in water flea population, then a decrease. These observations are independent of any changes in the heart rates, but involve the changes in the lake community. How could you account for the change in water flea populations?

Answer to Test Question

The initial increase in water flea numbers could be caused by an increase in unicellular algae on which they feed. The subsequent decrease could be caused by an increase in the size and appetite of the fish predators of the water fleas. It also could be caused by a decrease in the oxygen concentration of the water as the algae die and bacterial decay of their carcasses reduces the oxygen in the water.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1. Use chemicals specified for Variation 3.

VARIATION 4

The Effect of Vinegar on the Heart Rate of Daphnia

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS

Students will compare the heart rates of *Daphnia* before and after exposure to vinegar.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 5.0 mL 2.0% distilled vinegar in culture water
- ♥ 10 to 12 *Daphnia*

TEACHING TIPS

- Lowering an alkaline pH causes more oxygen to be released from some crustaceans' hemoglobin. The oxygen is released until a critical value is reached where the effect is reversed (Wolvekamp & Waterman, 1960).
- Lowering the pH mimics the metabolic effects of increasing the carbon dioxide concentration. For some crustaceans, the result is an initial increase in respiration rate followed by a decrease in respiration rate. For others, an initial decrease is followed by an initial increase (Wolvekamp & Waterman, 1960).

SAFETY PROCEDURES

Practice safe handling of acids. Add acid to water rather than water to acid.

Wear goggles and lab apron when working with acids.

Clean all spills immediately.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Prepare a 10.0% vinegar solution by adding 10.0 mL of distilled vinegar (5.0% acetic acid) to 90.0 mL of culture water.

HYPOTHESIS GENERATION

Question

What is the effect of vinegar on *Daphnia* heart rate?

Sample Hypothesis

The heart rate of *Daphnia* will decrease if they are exposed to vinegar.

Rationale

Acid rain has killed the animals in many lakes. Vinegar is an acid that may have the same effect as the acids of acid rain.

Sample Experimental Procedure

1. Repeat the Core Experiment with the 10.0% vinegar solution and unadulterated culture water.
2. Expose the *Daphnia* to various concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Effect of vinegar on *Daphnia* heart rates.

Student team	Unexposed (beats/minute)	Vinegar-exposed (beats/minute)
1	262	300
2	248	280
3	256	260
4	240	290
5	290	300
6	262	290
7	280	280
8	278	280
Mean	265	285

TEACHER'S NOTES

Interpretation

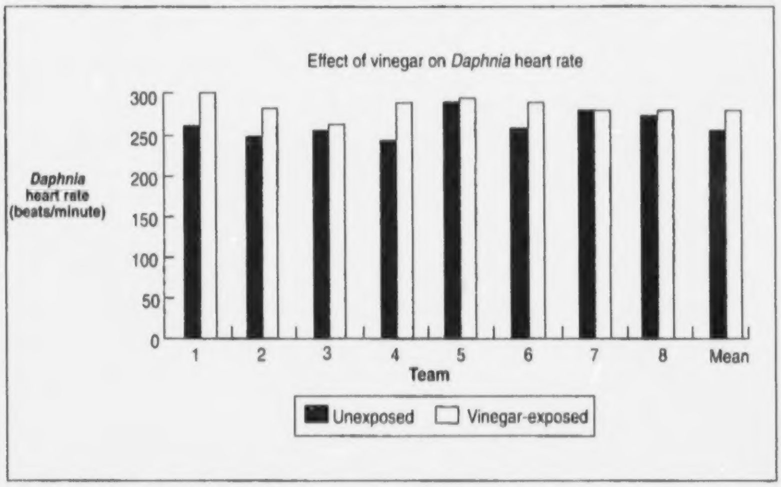
These data show increased heart rate with exposure to vinegar. These data do not support the hypothesis that heart rate would be depressed. An increase in heart rate still indicates that the water fleas are stressed by the treatment. In humans, an increase in heart rate occurs when there is an increased demand for oxygen, such as with exercise. Somehow, the vinegar may be reducing the available oxygen causing the water fleas' heart rate to increase.

Answer to Test Question

The water fleas were very sensitive to acid. Copepods may be even more sensitive. They probably have hemoglobin similar to water fleas and may have problems getting enough oxygen when the water is acidic. In addition, they may have problems with the calcium in their exoskeletons dissolving in acid.

TEACHING TIPS

- Provide students with syringes or pipettes, several test tubes or small beakers, and undiluted vinegar. Allow them to prepare their own dilutions.
- See Teaching Tips, Variation 4.



Graph H. The relationship of vinegar on *Daphnia* heart rate.

TEST QUESTION

Crustaceans belonging to the subclass containing water fleas have soft, pliable skeletons of chitin. Other planktonic crustaceans, like copepods and ostrapods, have calcareous plates reinforcing their chitin exoskeletons. Do you expect that water fleas or copepods will be more sensitive to acidification?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1. Use the chemicals in Variation 4.

VARIATION 5

The Effect of Different Concentrations of Vinegar on the Heart Rate of Daphnia

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Students will compare the effects of different vinegar concentrations on *Daphnia* heart rate.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 5.0 mL 10.0% distilled vinegar in culture water
- ♥ 5.0 mL 1.0% distilled vinegar in culture water
- ♥ 5.0 mL 0.1% distilled vinegar in culture water
- ♥ 5.0 mL 0.01% distilled vinegar in culture water

SAFETY PROCEDURES

See Safety Procedures, Variation 4.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Prepare the 10.0% vinegar solution by adding 10.0 mL of distilled vinegar (5.0%) to 90.0 mL of culture water.
- A 1.0% solution is made by adding 1.0 mL of the 10.0% solution to 9.0 mL of culture water.
- A 0.1% solution is made by adding 1.0 mL of the 1.0% solution to 9.0 mL of culture water.
- A 0.01% solution is made by adding 1.0 mL of the 0.1% solution to 9.0 mL of culture water.

HYPOTHESIS GENERATION

Question

What is the effect of varying concentrations of vinegar on *Daphnia* heart rate?

Sample Hypothesis

The heart rate of *Daphnia* decreases more with exposure to increasing concentrations of vinegar.

Rationale

Acid rain has killed the animals in many lakes. Vinegar is an acid that may have the same effect as the acids of acid rain. In the Core Experiment, the ethanol-stressed, sometimes dying, water fleas had reduced heart rates. Increasing the concentration of a toxin should increase the stress it creates in an animal.

Sample Experimental Procedure

1. Repeat the Core Experiment using these 4 concentrations of vinegar and unadulterated culture water.
2. Expose the *Daphnia* to the concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

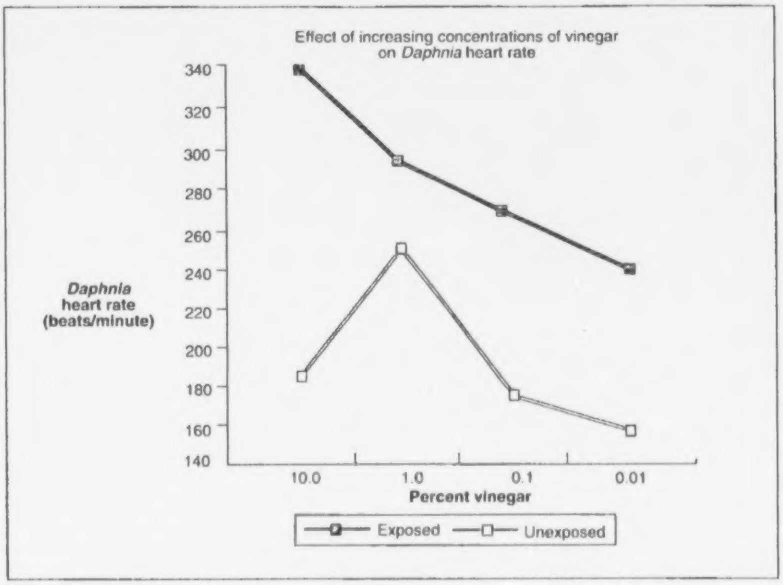
DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Effect of increasing concentrations of vinegar on the *Daphnia* heart rate. Values reported are the average of rates of 10 animals.

Percent vinegar	Exposed	Unexposed
10.0	340	184
1.0	288	250
0.1	270	174
0.01	240	153

TEACHER'S NOTES



Graph I. The effect of increasing vinegar concentrations on *Daphnia* heart rate.

Interpretation

These data show increasing heart rates with increasing vinegar concentration. These data support the hypothesis that heart rates would be increased and indicate that the water fleas are stressed by the treatment.

TEST QUESTION

Adding vinegar to water increases the hydrogen ion concentration, thus lowering the pH. What activities by man or nature could cause an increase in the hydrogen ion concentration of lakes?

Answer to Test Question

Anything that increases the carbon dioxide concentration of water could increase the hydrogen ion concentration. This would include decay of an algal bloom. Sulfur and nitrogen oxides are produced by the burning of fossil fuels and eruption of volcanoes that react with water in the air to form acids. Industrial spills also could introduce acids to waterways.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1. Use chemicals specified for Variation 5.

VARIATION 6

The Effect of Vinegar on the Phototactic Behavior of Daphnia

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will compare the phototactic responses of *Daphnia* exposed and not exposed to vinegar.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 20 *Daphnia*
- ♥ 2 large paper "backgrounds" divided evenly into dark and light sides
- ♥ 1 10-mL graduated cylinder of pipette with bulb
- ♥ 10.0 mL 2.0% solution of vinegar
- ♥ 10.0 mL culture water
- ♥ 2 petri dishes
- ♥ 2 pieces of filter paper
- ♥ 1 5-mL plastic, disposable pipette
- ♥ 2 15-cm squares of dark opaque plastic (garbage bag)

HYPOTHESIS GENERATION

Question

What is the effect of vinegar on the phototactic response of *Daphnia*?

Sample Hypothesis

The phototactic response of *Daphnia* will be affected if they are exposed to a weak solution of vinegar.

Rationale

If exposure to acid results in oxygen starvation, nervous system damage could result in long term disorientation.

Sample Experimental Procedure

1. Drain the water from 10 *Daphnia* by gently pipetting them onto a piece of filter paper.
2. Transfer them quickly to a petri dish containing 10.0 mL of 2.0% vinegar.
3. Repeat the same draining process for another 10 *Daphnia*, but transfer these water fleas to a petri dish with 10.0 mL of culture water.
4. Prepare 2 large paper "backgrounds" divided evenly into dark and light sides.
5. Set both dishes on the divided background so that half the dish is over light and half the dish is over dark.
6. Drape the dark, opaque plastic over the dark side of the dish. See Figure 3.

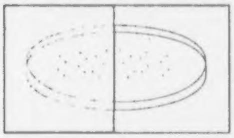


Figure 3. Experimental setup for phototactic behavior observation.

7. Every minute for 15 minutes, count the number of *Daphnia* in the light in each dish.
8. At the end of the 15 minutes, transfer them to the culture container reserved for specimens that have been stressed previously.
9. Calculate the total animal-minutes spent in the light in each container.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 7. Percent of *Daphnia* in the light during each minute of a 15-minute observation.

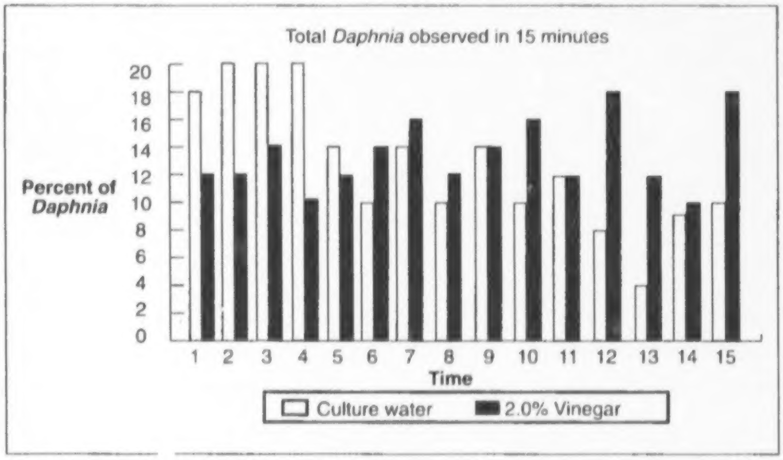
Time (minutes)	Culture water	2.0% Vinegar
1	18	12
2	20	12
3	20	14
4	20	10
5	14	12
6	10	14
7	14	16
8	10	12
9	14	14
10	10	16
11	12	12
12	8	18
13	4	12
14	9	8
15	8	18

(continued on p. 358)

TEACHER'S NOTES

(continued from p. 357)

Total the number of animals observed in the light throughout the 15 minutes. An appropriate analysis here would be Chi-Square Goodness-of-Fit with the hypothesis that there will be no difference in light preference; that is, that the total number of animal-minutes in the light will be the same in the culture water and in the vinegar.



Graph J. Percent *Daphnia* observed in light over a 15-minute period.

Interpretation

Nearly the same amount of time was spent in the light in both the treatment (acid) and in the control (culture water). If all the water fleas had spent the entire time in the light, there would have been 300 animal-minutes recorded for each petri dish. Nearly two-thirds of the possible animal-minutes were in the light (191/300 in the culture water and 200/300 in vinegar). No disorientation to the light is apparent when the water fleas are in 2.0% vinegar. Both vinegar and culture water *Daphnia* prefer the light.

TEST QUESTION

In this experiment *Daphnia* were found to prefer light to dark. Why is it advantageous for them to have the ability to orient to the light?

Answer to Test Question

Daphnia are filter feeders. Their primary food source is probably single-celled algae. The algae require light to photosynthesize. If the water fleas are attracted to light, they will be attracted to the area of the lake where their food source is most abundant.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have the visually impaired student collect data and observations of phototactic responses from the team and present in bar and line graphs as needed.

VARIATION 7

The Effect of Temperature on the Phototactic Behavior of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students will determine if temperature influences the phototactic behavior of *Daphnia*.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- ♥ ice
- ♥ 1 refrigerator
- ♥ 1 large paper "background" divided evenly into dark and light sides
- ♥ 4 15-cm squares of dark opaque plastic (garbage bag)

(continued on p. 359)

(continued from p. 358)

- 10-mL graduated cylinder of pipette with bulb
- 1 thermometer (°C)
- 4 petri dishes
- 40 *Daphnia*
- 4 culture dishes larger than the petri dishes to serve as water baths
- 2 water baths
- 1 ice bath *(optional)*
- 1 bright light source
- 2 pieces of filter paper

SAFETY PROCEDURES

Use caution when preparing and using the hot water bath.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Preheat 100.0 mL of culture water to 25°C and another 100.0 mL to 30°C. Maintain the temperatures with water baths.
- Chill 100.0 mL of culture water in the refrigerator or an ice bath to 5°C and another 100.0 mL to 15°C.

HYPOTHESIS GENERATION

Question

What is the effect of temperature on the phototactic behavior of *Daphnia*?

Sample Hypothesis

Water temperature will affect the phototactic behavior of *Daphnia*.

Rationale

During a bright sunny day, *Daphnia's* algal food source should be at the surface of the water that is slightly warmer than the water below it. Optimal temperature for enzyme activity is fairly narrow. *Daphnia* should be able to migrate to their optimal temperature independent of the light intensity.

Sample Experimental Procedure

1. Set up 4 petri dishes containing 100.0 mL water each at the following temperatures: 5°C, 15°C, 25°C, and 30°C.
2. Maintain the desired temperature in each petri dish by setting it in a larger dish with water of the same temperature.
3. Drain the water from 10 *Daphnia* by gently pipetting them onto filter paper. Quickly transfer them to a petri dish with 10.0 mL of water at the desired temperature.
4. Repeat the same draining process for another 10 *Daphnia*, but transfer these water fleas to a petri dish with 10.0 mL of water at another desired temperature. Continue this process until dishes of all desired temperatures are prepared.
5. Prepare 1 large plastic or laminated paper "background" divided evenly into dark and light sides to hold the 4 petri dishes.
6. See Sample Experimental Procedure Variation 6. Repeat Steps 6 and 7.
7. Return *Daphnia* to container of previously stressed organisms.
8. Calculate the total animal-minutes spent in the light in each container.

TEACHER'S NOTES

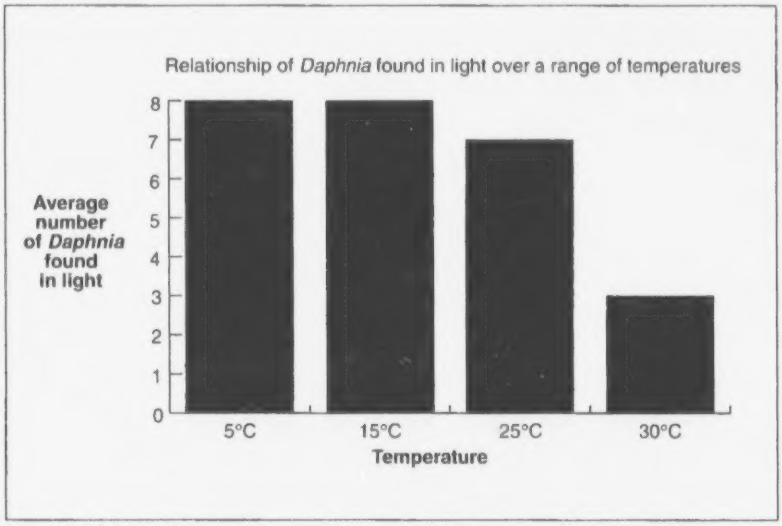
DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 8. Number of *Daphnia* in light at several temperatures. Ten *Daphnia* were placed in each container for 15 minutes.

Time	5°C	15°C	25°C	30°C
1	8	8	7	5
2	9	8	7	4
3	7	8	7	4
4	8	7	8	6
5	9	7	6	3
6	9	8	6	3
7	8	9	8	5
8	9	8	5	2
9	10	7	7	2
10	7	8	6	1
11	7	8	8	1
12	10	7	5	2
13	9	9	5	1
14	8	8	7	1
15	7	8	8	1

An appropriate analysis would be Chi-Square Goodness-of-Fit using the hypothesis that there will be no difference in the number of *Daphnia* in the light in water at different temperatures.



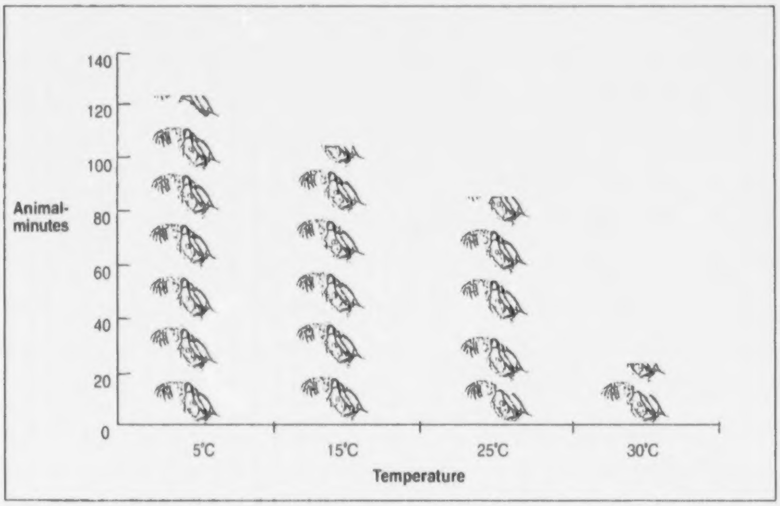
Graph K. Effect of varying temperatures on *Daphnia's* presence in the light.

Interpretation

The warmer the water, the fewer *Daphnia* were found in the light. There was a dramatic reduction in the number of *Daphnia* in the light between 25 and 30°C. These data support the hypothesis that orientation to light is influenced by water temperature.

TEST QUESTION

Compare the student data graphed below with the results of your experiment.



Graph L. Influence of temperature on the phototactic response of *Daphnia*.

Answer to Test Question

These students should find a similar trend in their water fleas' response to light at different temperatures.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide blind students with Talking Thermometers so they can record temperatures of various cultures.

VARIATION 8

The Effect of Seltzer Water on the Phototactic Behavior of Daphnia

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will compare the phototactic responses of *Daphnia* exposed to seltzer water with those of *Daphnia* not exposed to seltzer water.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- 1 large paper "background" divided evenly into dark and light sides.
- 10.0 mL seltzer water
- 10.0 mL culture water
- 1 1-mL disposable pipette
- 20 *Daphnia*
- 2 pieces of filter paper
- 2 petri dishes
- 2 15-cm squares of dark opaque plastic (garbage bag)
- 1 10-mL graduated cylinder

TEACHER'S NOTES

HYPOTHESIS GENERATION

Question

What is the effect of seltzer water on the phototactic behavior of *Daphnia*?

Sample Hypothesis

The phototactic responses of *Daphnia* will be affected if they are exposed to seltzer water.

Rationale

Students should provide their own rationale for the hypothesis.

Sample Experimental Procedure

1. Drain the water from 10 *Daphnia* by gently pipetting them onto filter paper. Quickly transfer them to a petri dish with 10.0 mL of 10% seltzer water.
2. Repeat the same draining process for another 10 *Daphnia*, but transfer these water fleas to a petri dish with 10.0 mL of culture water.
3. Prepare 1 large paper "background" divided evenly into dark and light sides.
4. Set both dishes on the divided background so that half the dish is over light and half the dish is over dark.
5. See Sample Experimental Procedure, Variation 6. Repeat Steps 6 to 9 with the exception of Step 8 where the draining process will remove seltzer water, not acid as described in Variation 6.

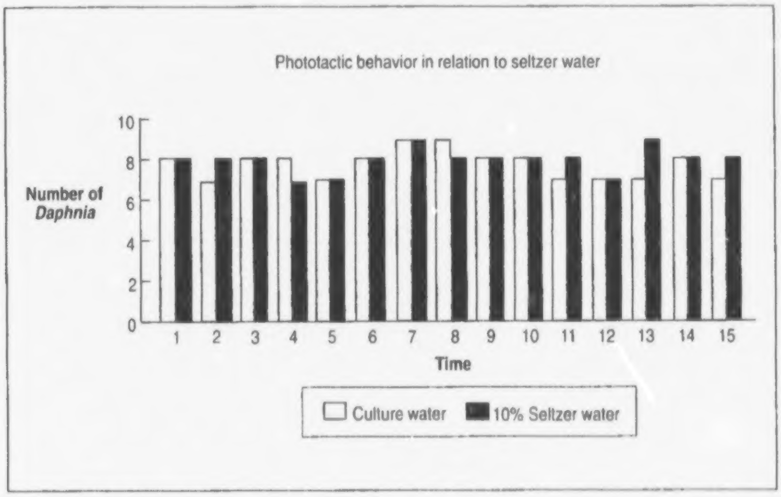
DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 9. Number of *Daphnia* in light when incubated in 10% seltzer water and in culture water. Ten *Daphnia* were in each container for 15 minutes.

Time	10% Seltzer water	Culture water
1	8	8
2	7	8
3	8	8
4	8	7
5	7	7
6	8	8
7	9	9
8	9	8
9	8	7
10	8	8
11	7	8
12	7	7
13	7	9
14	8	8
15	7	8

Total the number of animals observed in the light throughout the 15 minutes. An appropriate analysis here would be Chi-Square Goodness-of-Fit with the hypothesis that there will be no difference in light preference, that is, that the total number of animal-minutes in the light will be the same in the culture water and in the seltzer water.



Graph M. Effect of seltzer water on *Daphnia*.

TEST QUESTION

Although the phototactic response of *Daphnia* was not altered by exposure to seltzer water, the activity level was altered. Would you consider seltzer water a toxin? Explain.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 6.

VARIATION 9

The Effect of the Time of Day on the Phototactic Behavior of Daphnia

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS

Students will determine if the diurnal cycle of *Daphnia* influences their phototactic response.

HYPOTHESIS GENERATION

Question

What effect does time of day have on the phototactic response of *Daphnia*?

Interpretation

A decrease in activity of the *Daphnia* in the treatment was noticed, but there was no difference in the number of animal-minutes in the light in the treatment and in the culture water.

Answer to Test Question

Seltzer water could be considered a toxin for the *Daphnia* because they did not behave normally when exposed to it. The failure to measure a difference may mean that the correct response to detect the effect of seltzer water was not chosen.

TEACHING TIP

Students in two different biology classes could perform this variation and compare data. One class could be scheduled for the early morning hours, and the other for the late afternoon. The early class should plan to come in about 7 a.m.

TEACHER'S NOTES

Sample Hypothesis

Daphnia will show a stronger phototactic behavior in the early morning than in the late afternoon.

Rationale

During the early morning hours, *Daphnia* should be following their food source to the surface. In the heat of the afternoon or in the waning hours of light, they may retreat from the lighted surface, again following their food source.

Sample Experimental Procedure

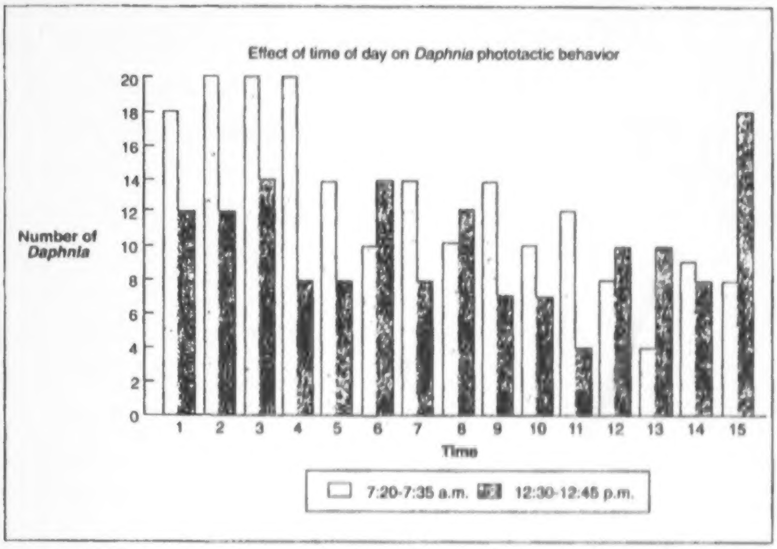
1. Drain the water from 20 *Daphnia* by gently pipetting them onto filter paper. Quickly transfer them to a petri dish with 10.0 mL of culture water.
2. Prepare 1 large paper "background" divided evenly into dark and light sides.
3. Set the dish on the divided background so that half the dish is over light and half is over dark.
4. Count the number of water fleas in the light in each dish for 15 minutes at 2 different times of the day, such as in the morning and at midday.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 10. Number of *Daphnia* in the light during each minute of a 15-minute observation at different times of day. There were 20 *Daphnia* in each observation dish.

Time (minutes)	**7:20 to 7:35 a.m.**	**12:30 to 12:45 p.m.**
1	18	12
2	20	12
3	20	14
4	20	8
5	14	8
6	10	14
7	14	8
8	10	12
9	14	7
10	10	7
11	12	4
12	8	10
13	4	10
14	9	8
15	8	18



Graph N. Effect of time of day on *Daphnia*'s phototactic behavior.

TEST QUESTION

Do *Daphnia* show a positive or negative phototactic response in the early morning? What evidence supports your answer?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 6.

VARIATION 10

The Effect of Phosphate-Containing Detergent on the Phototactic Behavior of Daphnia

Note to Teachers: *In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.*

SYNOPSIS

Students will compare the phototactic responses of *Daphnia* exposed to phosphate with those of *Daphnia* not exposed to phosphate.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- 1 large paper "background" divided evenly into dark and light sides
- 2.0% trisodium phosphate (TSP)

SAFETY PROCEDURES

See Safety Procedures, Variation 2.

Interpretation

In the early morning, 191 animal-minutes were spent in the light. In the early afternoon, only 152 animal-minutes were spent in the light. These data support the hypothesis. During the early morning hours, *Daphnia* should be following their food source to the surface. In the heat of the afternoon or in the waning hours of light, they may retreat from the lighted surface, again following their food source.

Answer to Test Question

Daphnia show a positive phototactic response in the early morning. If there were no phototactic response, one would have expected to find only 150 animal-minutes in the light. If the response were negative, one would have expected to find still fewer. The observation of 191 animal-minutes in the light supports a positive phototactic response.

TEACHING TIPS

See Teaching Tips, Variation 2.

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TEACHER'S NOTES

HYPOTHESIS GENERATION

Question

What is the effect of phosphate-containing detergent on the phototactic response of *Daphnia*?

Sample Hypothesis

The phototactic responses of *Daphnia* will be affected if they are exposed to phosphate-containing detergent.

Rationale

Phosphate is a recognized pollutant. It should be assumed to be harmful to the normal functioning of an organism.

Sample Experimental Procedure

1. Drain the water from 10 *Daphnia* by gently pipetting them onto filter paper. Quickly transfer them to a petri dish with 10.0 mL of 2.0% TSP.
2. Repeat the same draining process for another 10 *Daphnia*, but transfer these water fleas to a petri dish with 10.0 mL of culture water.
3. Prepare 1 large paper "background" divided evenly into dark and light sides.
4. Set both dishes on the divided background so that half the dish is over light and half the dish is over dark.
5. See Sample Experimental Procedure, Variation 6. Repeat Steps 6 to 9 with the exception of Step 8 where the draining process will remove TSP, not acid as described in Variation 6.

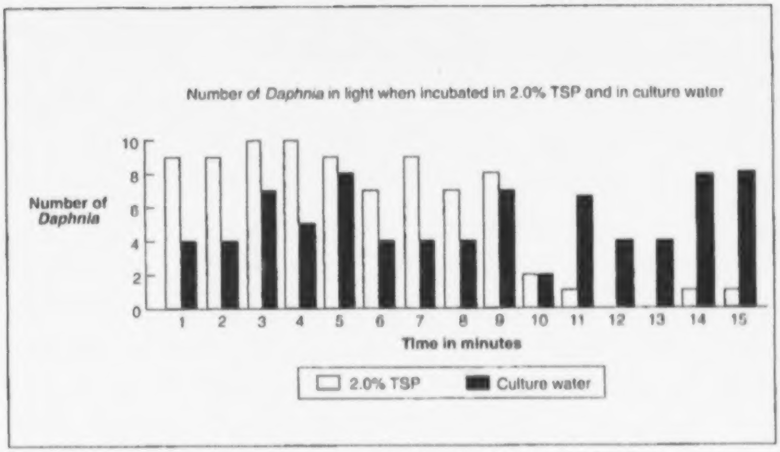
DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 11. Number of *Daphnia* in light when incubated in 2.0% TSP and in culture water. Ten *Daphnia* were in each container for 15 minutes.

Time	2.0% TSP	Culture water
1	9	4
2	9	4
3	10	7
4	10	5
5	9	8
6	7	4
7	9	4
8	7	4
9	8	7
10	2	2
11	1	7
12	0	4
13	0	4
14	1	4
15	1	4

An appropriate analysis would be Chi-Square Goodness-of-Fit using the hypothesis that there will be no difference in the number of *Daphnia* in the light in different solutions.



Graph O. The effect of phosphate on the phototactic behavior of *Daphnia.*

TEST QUESTION

Create graphs to display the following experimental results obtained by two different researchers.

Table 12a. Number of *Daphnia* in light when incubated in 2.0% TSP and in culture water. Ten *Daphnia* were in each container for 15 minutes.

Time	2.0% TSP	Culture water
1	9	8
2	9	7
3	10	8
4	10	9
5	9	7
6	7	7
7	9	7
8	7	7
9	8	8
10	2	8
11	1	7
12	0	6
13	0	8
14	1	8
15	1	8

Interpretation

More animal-minutes in the light were observed in the phosphate treatment (83) than in the culture water (72). These data support the hypothesis that phosphate pollution alters the phototactic response of *Daphnia.*

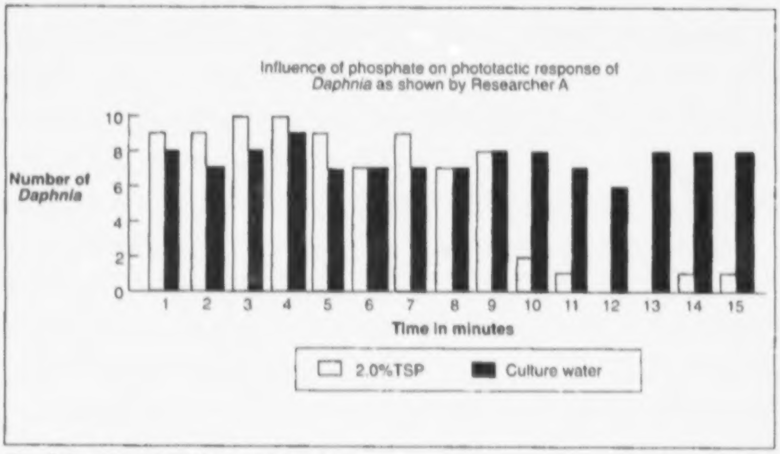
Answer to Test Question

Graphs P and Q. Influence of phosphate on phototactic response of *Daphnia* as found by two different researchers.

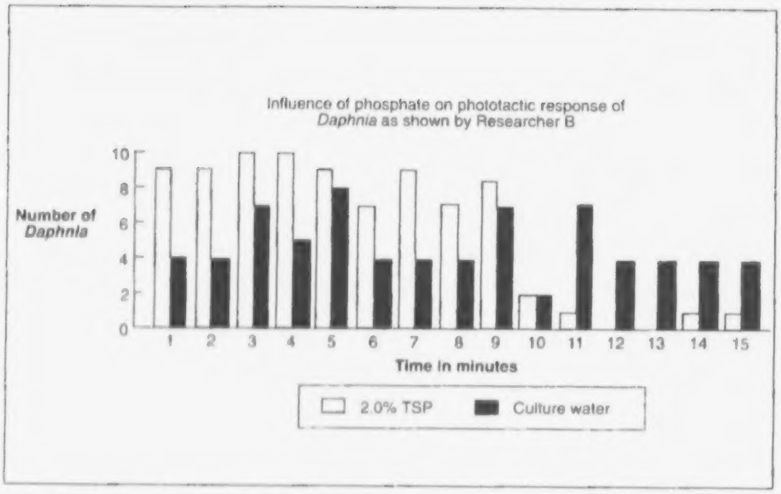
TEACHER'S NOTES

Table 12b.

Time	2.0% TSP	Culture water
1	9	4
2	9	4
3	10	7
4	10	5
5	9	8
6	7	4
7	9	4
8	7	4
9	8	7
10	2	2
11	1	7
12	0	4
13	0	4
14	1	4
15	1	4



Graph P. Researcher A's graphed results.



Graph Q. Researcher B's graphed results.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 6.

Interpretation

These results are the opposite. Group P shows a stronger, positive phototactic response in TSP than in culture water. Graph Q shows the stronger response in culture water. It is possible that Researcher A did the experiment at a different time of day or at a different temperature. The influence of these variables on the response was not tested.

NATURE'S EARLY WARNING SYSTEMS

Directions for Students

INTRODUCTION

The sun is barely up, and near the shore of a shallow lake there seem to be hundreds of identical little water fleas about 5 centimeters long. By early afternoon this bright sunny day, the fleas have nearly all disappeared. Where did they go? Are they really fleas? Could they bite you? Are they really all identical, or do you just need to look closer to recognize differences between them? Are they in all shallow lakes and ponds?

Some of these questions are answered easily. For instance, if the small fish have not eaten them and if the lake has not changed during the day, they are probably resting on the mud at the bottom in the early afternoon. They will be back to the surface later in the afternoon, as they follow a rhythm set by light reception and their biological clocks. They are not really fleas, even though they make a jerky, flea-like movement, and they will not bite you. A good meal for these crustaceans (*Daphnia)* is single-celled green algae, a few diatoms, and some bacteria. At some times of the year, they really are all alike. Under some conditions, *Daphnia* produce nearly identical offspring–at least, they're all daughters!

The last two questions are harder to answer. What accounts for the absence of an animal could be just that it was never there, or that conditions were not right for its survival when it arrived, or that conditions have changed since it arrived. According to wildlife officials, *Daphnia* are transported easily by birds. We also know that *Daphnia* are extremely sensitive to some toxins, such as low concentrations of industrial wastes or run-off from lawn care chemicals. They are more sensitive to most materials that will harm them than are the fish who feast on them. Their sensitivity, small size, and ease of culture make them excellent subjects for assaying the toxicity of waters before other organisms show any signs of damage. You will explore the effect of a toxic material on the physiology of *Daphnia* at levels too low to be lethal. See Figure 1.

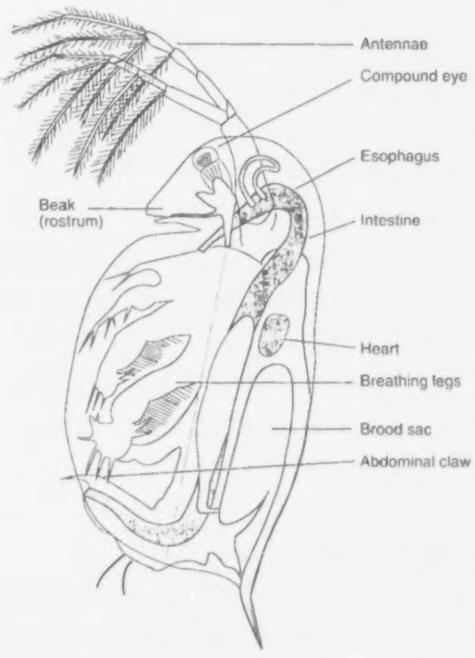


Figure 1. A species of *Daphnia* with several internal structures identified. (Used with permission. Collins, K. & B. (1985). *Daphnia* — A Handy Guide. *The American Biology Teacher, 47(50),* 299.

OBJECTIVES

At the end of this lab, you should be able to:

- Describe possible physiological or behavioral effects of sublethal doses of ethanol on *Daphnia.*

SAFETY PROCEDURES

- Wear goggles and a lab apron when working with chemicals.
- Work away from any open flames when using ethanol, especially when wearing gloves.
- Wear latex gloves when working with ethanol. It can be absorbed through your skin.
- Wash your hands after all work is completed.
- Do not consume ethanol.
- Dispose of broken glassware in appropriate containers.
- Use KittyLitter™ to absorb any large spills. Sweep up the KittyLitter™ after it has absorbed the spilled material.

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MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- 10 *Daphnia*
- 1 culture dish
- 1 50-mL jar or beaker
- 1 wide-bore pipette
- 2 1-mL plastic, disposable pipettes
- 1 compound microscope
- 1 depression slide and cover slip
- 1 lab journal
- 1 pencil
- 1 clock with second hand or a stopwatch
- 1 calculator
- several paper towels or filter paper
- 2 pairs of latex gloves *(optional)*
- 25.0 mL of 2.0% ethanol solution

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of chemical toxicity and *Daphnia*. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. What makes this question an interesting or important topic for investigation?
3. What is the variable in your experiment?
4. What other variables will you need to control?
5. Why is controlling variables important?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. Combine your results with those of all your classmates. Construct a graph showing the heart rates of *Daphnia* exposed to ethanol and not exposed to ethanol.
2. Do these data support or refute your hypothesis? Explain.
3. If half of the ethanol-treated animals had depressed heart rates and the other half did not, would you accept or reject your hypothesis? Explain.
4. Suppose all of the animals showed a 20% depression in heart rates when exposed to ethanol, while those tested from another lake by students across town showed a 10% depression. How could you explain the difference?
5. Propose a test of your explanation to the question above.

DESIGN AND VARIATIONS OF THE CORE EXPERIMENT

After collecting and analyzing these data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for experiments you could do next. Think of questions that occurred to you as you conducted the Core Experiment on Daphnia and their role as environmental indicators. What quantifiable experiments could be done based on the observations you made? Questions other students have studied include the following:

- What is the effect of ethanol on *Daphnia* heart rates?
- What is the effect of varying concentrations of ethanol on *Daphnia* heart rates?
- What is the effect of phosphate on *Daphnia* heart rates?
- What is the effect of varying concentrations of phosphate on *Daphnia* heart rates?
- What is the effect of vinegar on *Daphnia* heart rates?
- What is the effect of varying concentrations of vinegar on *Daphnia* heart rates?
- What is the effect of vinegar on the phototactic response of *Daphnia*?
- What is the effect of temperature on the phototactic behavior of *Daphnia*?
- What is the effect of seltzer water on the phototactic behavior of *Daphnia*?
- What is the effect of phosphate-containing detergent on the phototactic response of *Daphnia*?

Appendices

Appendix A: Writing Format for *Student Research*

Directions for Teachers

Synopsis for Core Experiment summarizes the main concepts of the Core Experiment.

Appropriate Biology Level indicates the appropriate biology level for the activity, such as beginning, intermediate, or advanced.

Objectives for Core Experiment describes measurable student outcomes of concepts, content, process, skills, and attitudes that students should understand and be able to explain at the conclusion of the activity.

Getting Ready includes:
- *Length of Lab*...suggested time in minutes and number of days to conduct the activity.
- *Materials Needed*...for a class of 24 students.
- *Preparation Time Required*...an estimated time for preparing the activity.
- *Directions for Setting Up the Lab*...directions for preparation of solutions, collection of materials, and construction of lab apparatus.

Safety Procedures includes lab-specific safety considerations.

Teacher Background includes:
- *Content Information*...non-technically written scientific content information.
- *Pedagogical Information*...list of common student misconceptions that are lab-specific with the correct concepts.

Teaching Tips includes lab-specific trouble spots that anticipate where students may have problems, alternative equipment and material, appropriate time of the year to conduct the lab, and where to purchase lab-specific materials.

Instructional Procedures for the Core Experiment includes:
- *Introduction*...strategies to introduce the lab to students and pique their interest such as demonstration, questions, or discrepant events. Instructions assist the teacher in providing enough content background information to the students with leading questions to facilitate the development of their own hypotheses and lab designs.
- *Hypothesis Generation*...strategies that the teacher can use to facilitate the development of good student hypotheses. A sample question, hypothesis, and rationale for that hypothesis are included as a guide.
- *Procedure*...a sample procedure for conducting the experiment for the Sample Hypothesis.
- *Data Analysis and Interpretation*...a sample analysis of student data and a discussion of that data for further understanding.
- *Test Questions* and *Answers to Test Questions*...questions with answers about the lab topic.

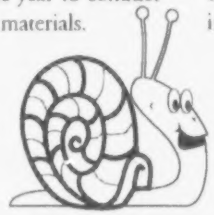
Student Design of the Next Experiment includes questions that assist students in developing other questions that may lead to the design of another experiment.

Suggested Modifications for Students Who Are Exceptional includes lab-specific suggestions for modifying the lab for students with special needs.

Answers to Questions and Analysis on Student Page provides answers to sections of the **Directions for Student** pages.

References and Suggested Readings contains sources specifically cited and quoted in the lab, as well as sources for further reading on the topic.

Variations of the Core Experiment includes up to 10 variations of the Core Experiment. Only information that is unique to each Variation of the Core Experiment is found in this section.



... Continued Appendix A: Writing Format

Directions for Students

These directions are written so that students are not required to write answers on these pages.

Introduction includes major questions that focus the student on the lab. This section is not intended to provide answers to the lab or information on how to design the lab.

Objectives describes the expected student outcomes.

Safety Notes contains lab-specific and student-oriented safety considerations.

Materials Needed lists the materials needed for one student team.

Student Literature Search Summary with References provides suggestions for resources for student research of the literature.

Hypothesis Generation contains directions to help students design a lab-specific hypothesis.

Plan of Investigation asks students to design an experiment based upon the material presented in the teacher introduction section.

Questions and Analysis includes questions that help students analyze the quantitative data they have gathered. Answers are included in the teacher section.

Design of Variations of Core Experiment provides questions related to the Variations of the Core Experiment to assist students in designing additional experiments.

Appendix B:

Resources on NABT Web Site

For information on how to contact individuals who have used these activities, access the NABT Web site *www.nabt.org*. The site contains the activities and E-mails of scientists, writers, and field testers of the *Student Research* activities, where available.

Appendix C: Standard Microbiological Practices and Aseptic Techniques

Standard Microbiological Practices

(Adapted from *CDC-NIH Biosafety in Microbiological and Biomedical Laboratories*, 2nd ed., May 1988, 11-12.)

1. Access to the laboratory should be limited or restricted at the laboratory director's discretion when experiments are in progress.
2. Decontaminate work surfaces once a day and after any spill of viable material. It is prudent to have students wipe the benches down with 10 percent bleach (1 volume of bleach plus 9 volumes of water from the tap) or disinfectant at the beginning and end of each class or session. Make sure no student is allergic to bleach before using and that gloves are worn when using the bleach.
3. Decontaminate all contaminated liquid or solid wastes before disposal. This is done by steam-sterilizing in an autoclave for 30 minutes at 15 pounds per square inch (psi) of pressure at 121°C. When cool, the wastes are ready for disposal. An alternate method of sterilization is to soak wastes in 10 percent bleach for one hour, then rinse before disposal.
4. Mouth pipetting is prohibited.
5. If you have any cuts on exposed hands or arms, be sure to wear gloves or do not handle DNAs or cells.
6. Do not eat, drink, smoke, or apply cosmetics in the work area. Food may be stored only in designated cabinets or refrigerators located outside the work area.
7. Lab participants should wash their hands both before and after handling viable materials and before leaving the laboratory.
8. Perform all procedures carefully to minimize the danger of aerosols. For example, do not force the last drop of liquid from a pipette. Place the pipette tip in the receiving vessel close to the liquid layer, then release the last drop.
9. Wear laboratory coats, gowns, or uniforms to prevent contamination or soiling of street clothes. These can be laundered in hot water with soap and bleach.

Special Practices

1. When autoclaving materials for disposal, loosen bottle caps and open the bags so steam can circulate to prevent the buildup of steam pressure and the possibility of explosion. If decontamination is to take place away from the laboratory, tightly seal contaminated materials in a durable, leak-proof container for transport. Autoclave for 30 minutes to be sure that all materials have been heated long enough to destroy spores and other contaminants.
2. Check with the school's engineer concerning the building's insect and rodent control program. Do not conduct any rDNA experiments without such a program. Put away all materials when not in use; insects and rodents may be attracted to spillage from contaminated materials.

Containment Equipment

Special containment equipment generally is not required for manipulations. A biosafety cabinet is not necessary.

Laboratory Facilities

1. The laboratory should be designed so that it can be cleaned easily.
2. Bench tops should be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture should be sturdy with the spaces between benches, cabinets, and equipment accessible for cleaning.
4. Each laboratory should have a sink for handwashing.
5. If the laboratory has windows that open, they should be fitted with screens.
6. Safety equipment should include a first-aid kit, fire blanket, all-purpose fire extinguisher, eyewash station, shower, and lab aprons for each student.
7. Each student should have his/her own safety goggles. Safety goggles should not be shared.
8. The laboratory must provide adequate work space for each student. Space should be available for all students to conduct the experiment simultaneously.

Aseptic Techniques

Remember! YOU are the major source of contamination. Hands, face, hair, clothing, the outsides of all objects—and even the air—contain microbes that can grow on the culture media. The following rules apply to teachers and students who work in the lab:

1. **Use sterile media, vessels & tools.**
   Purchase or prepare sterile culture media. You may also need to have sterile additives, pipettes, cylinders, flasks, or bottles and sterile deionized or distilled water for diluting. Sterile media poured into non-sterile vessels will be contaminated. Check the material to determine the best sterilization method.
2. **Keep hands far from the working ends of tools & vessels.**
   Most pipettes have a "double line" of safety. You can easily hold and operate a pipette upstream of this marking. Do not touch the tip or column of the pipette with any object, even hands or the bench top. If something may have touched the pipette, use a fresh, sterile one. Many suppliers package pipettes individually.
3. **Keep the work area neat.**
   A properly organized work area helps to keep the process aseptic. Items can be moved so that they are nearby when needed or out of the way so they do not accidentally become contaminated.
4. **Only uncover vessels when conducting a transfer.**
   Flame caps and necks of vessels before and after conducting a transfer. Lift the lid of the petri plate as seldom and at as shallow an angle as possible. Bacteria and mold spores are in the air. Culture media are a source of nutrition for these airborne contaminants. After some practice, pipetting can be done easily while holding the vessel cap open end down.
5. **Hold vessels as horizontally as possible when making a transfer.**
6. **Do not pass hands or arms over open vessels, caps or tools.**
   Hands, face, hair, arms, and sleeves naturally and normally harbor microbes, which can contaminate the experimental culture. Keep hands out of pockets and away from face and hair while working.
7. **Do not pipette out the last drop.**
   When the last drop in a pipette is blown out, large amounts of aerosols and droplets are produced.

Reprinted with permission from Horn, T.M. (1993). *Working with DNA & Bacteria in Precollege Science Classrooms.* Reston, VA: NABT.

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Appendix D: Tips for Conducting Multiple Student Research Activities

Some Tips for Sanity

This is not an exhaustive list, but may save you some time and increase your students' success in research.

- Take the time to insure that your students have selected a "doable" project.
- Have your students work with a mentor. It is impossible for one teacher to handle multiple topics and activities effectively.
- Have students use one, but no more than two of the *Student Research* activities. This will allow you to make optimum use of equipment, resources and your time.
- Contact and enlist the assistance of resource people via E-mail. Make them aware that your students will be contacting them directly for assistance.
- Work with other disciplines, such as the English, Computer, and Mathematics Departments. If planned in advance, these departments can utilize the skills and knowledge that they teach your students. For example, the Mathematics Department can assist with the correct methods for data analysis, statistics, and proper display of data. The English Department can assist with writing the scientific report in correct, clear grammatical form and style. Have the English Department clarify to the student that this is not a research paper as written for the typical English class, but a technical scientific paper to report the results of their laboratory research.
- Enlist the help of parents and make them aware of the research their child is doing.
- Set deadlines and keep students to them.
- Establish a Scientific Review Committee to review student topics, research plans, and research and to assist with necessary paperwork for use of organisms and human subjects.
- Work with the librarian to develop and strengthen student literary search skills. Keep in touch with the librarian on student progress with their literary searches in the library and on the Web.
- Consider group experiments to maximize time and equipment.
- Have students collaborate and update each other on a regular basis on their specific research.

Appendix E: Competitions for Student Research Activities

Students may wish to enter their work into a competition. There are many competitions that the students may enter including their own school, regional, and state science fairs. Many professional societies, private industries, and government institutions conduct student science competitions. Some major competitions that provide opportunities for students to present their projects are:

American Junior Academy of Science and individual *State Junior Academy of Science*, American Association for the Advancement of Science, 1200 New York Ave., NW, Washington, DC 20005, 202.326.6400.

International Science and Engineering Fair, Science Service, 1719 N Street, NW, Washington, DC 20036, 202.785.2255, *www.sciserv.org/iisef.htm.*

Intel Talent Search, Science Service, 1719 N Street, NW, Washington, DC 20036, 202.785.2255, *www.sciserv.org/stsl.*

The National Junior Science and Humanities Symposium (JSHS), The Academy of Applied Science, PO Box 2934, Concord, NH 03302-2934, 603.228.4520, FAX 603.228.4730, E-mail: *cousens@jshs.org.*

Appendix F: *Student Research* Evaluation Form

Please return the following evaluation form to NABT so that we may evaluate how the *Student Research* activities are implemented and their success with students to:

Kathy Frame, NABT Director of Education
12030 Sunrise Valley Drive, Reston Plaza One, Suite 110, Reston, VA 20191
Telephone: 703.264.9696; E-mail: *kframe@capaccess.org*. This form is available on the NABT Web site at *www.nabt.org*.

INSTRUCTOR: OVERALL REACTION TO THE CORE ACTIVITY AND VARIATIONS
Title of activity used:
Topic or unit where the activity was used:
Course title(s): **Subject title(s):**
Grade level(s): **Number of students:**

Check the appropriate response)

		1	2	3	4	5	
1. Overall, do you consider the activity a success in your classroom?	successful						failure
2. Did you use each part of this activity the way it was written?	exactly						highly modified
—core activity	exactly						highly modified
—variation(s) of core activity	exactly						highly modified
3. Did you find the core activity							
—easy to use?	very easy to use						difficult to use
— lengthy?	too long						too short
—original?	very original						not original
Comments:							
4. Did you find the variations		1	2	3	4	5	
—easy to use?	very easy to use						difficult to use
—lengthy?	too long						too short
—original?	original						not original
Comments:							
5. Was the overall design of this activity?	well organized						poorly organized
6. How did you implement the activity with students?	individually						as a class
Please explain:							
7. Does the activity fit into the curriculum?	easily						not easily
8. Could it be used with several topics of study in biology?	very flexible						limited in use
Are there additional topics to those covered in the classroom textbook? If so, please state.							
9. Does it allow for crossing through the curriculum?	interdisciplinary						limited to science
Please make suggestions as to how it could be used in conjunction with other subjects.							
10. Would you use this activity again?	definitely						never
If you responded 3, 4, or 5, how would you modify it so that you would use it?							
11. Would you encourage other teachers to use it?				Yes	No		
		1	2	3	4	5	
12. Will the typical high school biology teacher be able to use this activity?	easily						with difficulty
13. Were illustrations and graphs/charts:	easy to understand						confusing
	clearly labeled						poorly labeled
STUDENT RESPONSE TO THE CORE ACTIVITY AND VARIATIONS							
14. After completing the activity, did students want to learn more about the concepts of the activity?				Yes	No		
		1	2	3	4	5	
15. Were the students enthusiastic when responding to the activity?	very enthusiastic						not enthusiastic
16. Did students find the activity:	interesting						boring
	relevant to life						irrelevant to life
	too challenging						not challenging
17. Did this activity make it easy to motivate students to study the topic?	easy						difficult
18. Does the activity appeal to all students across cultures, gender, and socioeconomic levels?				Yes	No		
Comments:							
19. Did the activity help to develop students' skills in:		1	2	3	4	5	
—working with others?	useful						not useful
—developing hypotheses?	useful						not useful
—implementing lab design?	useful						not useful
—understanding graphs?	useful						not useful
—drawing graphs?	useful						not useful
—drawing conclusions from data?	useful						not useful
—thinking creatively, critically?	useful						not useful
—focusing on a task?	useful						not useful
20. Were there any particular difficulties, such as student frustration or difficulty with investigative science?				Yes	No		
If so, please comment briefly.							
21. Does the activity lend itself to more lab investigations?				Yes	No		

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